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Print ISSN: 0973-9122 Electronic - ISSN: 0973-9130
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Website: www.ijfmt.com

Editor
Dr. R.K. Sharma
Institute of Medico-Legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)

Printed, published and owned by
Dr. R.K. Sharma
Institute of Medico-Legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)

Published at
Institute of Medico-Legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)
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Socio-Demographic Profile of Fatal Poisoning in a Tertiary Care Teaching Hospital of Coimbatore District-A Retrospective Study

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Abstract

Background: Poisoning poses a major public health problem mainly in developing countries. Because of the rapid industrialization and advancement in agricultural sector, fatality by poisoning also increases day by day. The aim of this study is to determine the socio-demographic profile of fatal poisoning in Coimbatore District. Methods: This retrospective study was carried out in Department of Forensic Medicine, Coimbatore Medical College, Coimbatore, Tamil Nadu with data obtained from fatal poisoning cases brought for postmortem examination from January 2016 to December 2016. Identification of specific poisons were done with chemical examiner’s report. All collected data were analyzed in various possible aspects in the prepared proforma. Results: During the study period the total number of medico legal autopsies conducted were 3675 out of which fatal cases due to poisoning were 600(16.33%) cases. The study showed that the incidence of poisoning was more common among married men ie.303 cases (50.5%) out of 600 total number of poison cases. The incidence was more common in the age group 31 – 40 years in males (86 cases) which formed 14.3 % and in females it is 21-30 years (65 cases) which formed 10.8%. When we observed the religion wise distribution, Hindu males out numbered 369 out of 600 cases which formed 61.5%.

Conclusion: Morbidity and mortality due to poisoning can be minimized by health education, early referral, establishment of toxicological units for detection of specific poisons and appropriate guidance for proper management of poisoning cases at hospitals and primary health care centers.

Key words: Socio-demographic profile; poison; autopsy

Introduction

Poisons were known to antiquity. References to the poisons were found in ancient records worldwide. In prehistoric periods, there were professional poisoners. Orfila, who in nineteenth century brought precise chemical methods in toxicology is considered as father of Modern Toxicology. The first treatise on Indian Medicine was the Agnivesa Charaka Samhita written in seventh century BC. The first textbook on poisons was written by Mathew Joseph Orfila in 1814.

Because of the rapid industrialization and advancement in agricultural sector, the incidence of poisoning is spreading like a wild fire. The chemical substances that are developed to save the agricultural products from rodents and pests act as a double edged sword to mankind. Though there are advancement in medical research and treatment, death due to poisoning still remains in the higher side and it keeps on increasing day by day.

Death due to poisoning is significant medically, legally and socially. Fatality by poisons may be suicidal, homicidal or accidental. Suicidal poisoning is more

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common. Accidental poisoning occurs in children less than 5 years of age. Homicide by poison is rare. WHO estimated that approximately 3 million poisoning cases occur worldwide of which 22, 00,000 deaths occur per year. More than 90% of the cases are being reported from developing countries like India and Srilanka.

The aim of this study is to determine the socio-demographic profile of fatal poisoning in and around the Coimbatore city.

**Materials and Methods**

This retrospective study was carried out at the Department of Forensic Medicine and Toxicology, Coimbatore Medical College, Tamilnadu. Details were collected from the history of the case, treatment history and records, inquest papers, autopsy reports and chemical examiners report. Details of fatal poisoning cases brought for postmortem examinations from January 2016 to December 2016 were collected.

We used standard proforma to obtain data from the records to ensure consistency for the whole sample. Information collected includes age, sex, place of death, religion, marital status, medical treatment given, manner of death and chemical analyzer’s report. The collected data was statistically analyzed.

**Results**

This study revealed that a total of 3675 medico legal autopsies were conducted from 1st January 2016 to 31st December 2016 of which poisoning cases constituted 600 (16.3%) (Table 1)

Fatality in males outnumbered the females. Male female ratio being 2.03:1(Table 2) Out of total 600 cases, number of deaths in males is 401(66.8%), in females 198(33%) and in transgender 1(0.001%). Incidence of death by poisoning is more in the month of January 2016, 61 cases (10.2%) followed by 56 cases in February (9.3%) and in April 55 cases (9.16%)(Table 2)

In males, incidence of death by poisoning is more 86 cases in the age group 31-40 (14.3%) followed by 80 cases in age group 21-30 (13.3%),78 cases in age group 41-50 (13%), 69 cases in age group 51-60 (11.5%) , 34 cases in age group 61-70 (5.7%) , 26 cases in age group more than 70 years (4.3%), 24 cases in the age group less than 11-20 (4%) and 4 cases in the age below 10 years(0.6%) (Table 3)

In females, incidence of death by poisoning is more 65 cases in the age group 21-30 (10.8%) followed by 38 cases in the age group 31-40 (6.3%), 22 cases in age groups 51-60 and 61-70 (3.7%), 21 cases in age group 11-20 (3.5%),20 cases in the age group 41-50 (3.3%), 10 cases in age group more than 70 years (1.7%) and nil cases in the age group less than 10 years (Table 3)

In the present study, most of the cases were suicidal 596 cases (99.3%) followed by 4 accidental deaths(0.7%) Accidental poisoning occurs in children less than 10 years of age. There were no homicidal poisoning reported during the study period (Table 6)

**Discussion**

Whatever may be the manner of death, any unnatural death is a tragedy which wastes the precious human life and resources. In the present study male poisoning deaths are more than twice the female deaths with male to female ratio of 2:1. This is consistent with the results in the study conducted by A K Kapoor et al. This is supported by a similar study conducted by A K Batra et al which revealed male poisoning deaths more than twice the female deaths with male to female ratio of 1.0:0.49 which is maintained in more or less same in all age groups., B.R.Sharma et al in which males outnumbered the females, the male: female ratio being 2:1, Dr Kartik Prajapati et al which showed the male poisoning deaths 1.33 times more than the female deaths with a male to female ratio of 1.8:1, Alakesh Halder et al, Dr.S.S.Sandhu et al in which males are most affected than females in deaths by poisoning i.e
75.5%, B.D.Gupta et al⁷ in his study reported that males were prone to death by poisoning (62.1%) compared to females (37.9%) , Rajani V.Bhagora et al⁸ , in this study, poisoning death cases were higher as (57.58%) in males than in female deaths (42.42%). In their study, Dr.S.K.Chaudhary et al⁹ reported that the death due to poisoning in male population was 1.36 times more in comparison to female population with male to female ratio of 1:0.73, B.Suresh Kumar Shetty et al¹⁰ in their study observed that males outnumbered females constituting 69.2% (n=90) and 30.8% (n=40) of cases respectively with a male-female ratio of 2.3:1. In our study the most affected age group of fatal poisoning is 31-40 in males and 21 -30 years in females which is supported by a similar studies done by A K Batra et al² Dr.S.S.Sandhu et al⁶, B.D.Gupta et al⁷, Rajani V.Bhagora et al⁸, V.Koulapur et al¹¹ whom in their studies have reported that the fatal poisoning is more in third and fourth decade whereas it is less common in extremes of ages. This study revealed the maximum number of fatal poisoning occurred in married men. This finding is supported by the similar studies conducted by A K Batra et al², Dr.Kartik Prajapati et al⁴, Dr.S.S.Sandhu et al⁶ who reported that married persons (both sexes combined) outnumber the unmarried persons and constitute for over 62%, 62% and 57.6% deaths respectively. Dr.Virendar Singh et al¹² also observed that more married men (61.28%) were victims than unmarried men in his study. In our study, the incidence of death by poisoning is more in January 61 cases (10.2%) followed by higher incidence in February 56 cases (9.3%) and April 55 cases (9.16%). This is supported by a similar study by Kondrostami et al¹³ which reported that spring and autumn were the most troubling seasons regarding suicidal deaths. In the study conducted by A.K.Kapoor¹ maximum incidence of suspected poisoning admissions were recorded in the summer season comprising of 120 cases (39.9%) followed by monsoon season (32.6%), while for poisoning deaths, a total of 86 cases were recorded in the rainy season (41.9%), followed by summer (36.6%). Both in suspected poisoning cases and suspected poisoning deaths, a minimum incidence was noted in the winter months. In April 2003, a maximum of 34 cases of suspected poisoning were admitted whereas in August 03, a maximum of 28 poisoning deaths were recorded. In a study by A K Batra et al², these peaks were noted in mid-monsoon months of August and September, probably due to socio-economic reasons like monsoon-dependent cultivation practice, agriculture-based economy, crop failures, exorbitant rates of interest and indebtedness to private usuries, financial crisis, hunger, denied minimum wages and increased work and labor pressure in rainy season which lead to constant anxiety coupled with an easy availability of insecticidal poisons, as it is purchased and kept at forms and houses for use when the agricultural activities are at its peak. In the study done by Dr.S.Chaudhary et al⁹ maximum number of poisoning deaths were observed in month of May (13%) followed by month of March (11%) but statistically no seasonal trend is observed in the total number of events. Most of the poisoning deaths in the study conducted by B.Suresh Kumar Shetty et al¹⁰ were reported during the rainy and summer seasons. In a study conducted in Singapore (14), which has two monsoon seasons, the peaks for Indian suicides were noted in April, September and November. In the present study, poison deaths were highest in Hindus, 560 cases out of 600 (93%) followed by 24 cases in Christians (4%) and 16 cases in Muslims (2.6%). This is supported by similar studies (4;7;9) where the maximum number of deaths by poisoning were seen in Hindus when compared to other religions. Incidence of fatal poisoning was more in Hindu people (92.40%) as compared to Muslim (07.60%) in a study conducted by Dr.Kartik Prajapati et al⁴, B.D.Gupta et al⁷ concluded that most of the victims were Hindus, which can be explained by the fact that major population of India is Hindu. Dr.S.Chaudhary et al⁹ in his study reported that the incidence of fatal poisoning was more in Hindus (89.90%) as compared to Muslims (10.10%). In our study, the percentage of cases on manner of death is revealed as suicidal deaths 99.3% (596 cases), accidental in 0.7% (4 cases) and no homicidal cases. B.Suresh Kumar Shetty et al¹⁰ in their study stated that intentional self-poisoning constituted 79.2% (n=103) of these deaths, followed by accidental consumption of poison (19.2%). No case of homicidal poisoning death was reported during the study period. In two cases the manner of death remained undetermined. This report from our study is further supported by the study conducted by Tanuj Kanchan et al¹⁵ in which manner of death was reported as suicidal in 92.9% cases (n = 13) and accidental in one case (7.1%).

Conflict of Interest: None
Source of Support: Self

Ethical Clearance: Ethical clearance and funding were not necessary as it was a retrospective study which included only collection of data.

Table 1 – Number of Postmortems conducted in 2016

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<th>Murder</th>
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Table 2 - Sex and month wise distribution of cases

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Impact of Pilates Training versus Progressive Muscle Relaxation Technique on Quality of Life in Menopausal Women- A Comparative Study

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Abstract

Background: Menopausal women shows common symptoms which includes night sweats and hot flushes, heart distress, disturbances in sleep, depression, irritability, anxiousness, weight changes, physical and mental fatigue, joint and muscle pain. This affects the Quality Of Life of the Menopausal women. Pilates Training has proven beneficial for enhancing Quality Of Life in menopausal women but there is paucity of evidence of effect of Progressive Muscle Relaxation Technique in Menopausal women on QOL. The research is an initiative study that focuses on the effectiveness of PMRT on the various aspects of Quality Of Life of the menopausal women and comparison of the Pilates training and PMRT in order to enhance the QOL of participants.

Aim and Objective: Effect of Pilates training & Progressive Muscle Relaxation Technique on menopausal women’s Quality Of Life and comparison of both the technique.

Methods: 42 participants aged more than 45 years will be randomly categorized in two groups: Group A (n= 21) and Group B (n=21). The study duration will be of 6 months. The pre and post Intervention will be taken from both the group and data will be analyzed with the help of main outcome measure which is Menopause specific Quality Of Life (MENQOL).

Result- The expected result would include the details about whether the measures are effective for improving the quality of life in menopausal women, as well as the comparison of both the interventions. Data will be analyzed using paired t-test.

Conclusion- It will be published after results are analyzed

Keywords- Menopausal women, Quality Of Life, Pilates, Progressive Muscle Relaxation Technique.

Introduction

The concept of menopause is a complete cessation of ovarian function¹. Mean age for Indian women in menopause is 44 years. The symptoms peak between 45 to 55 years and decrease in severity after 55 years².

Most common symptoms of menopause include muscle and joint pain, slow feeling, poor memory, lower back pain and trouble sleeping. The vasomotor and emotional domains reported less often compared with the physical and psychological domains². About 75 per cent of women are reported to experience acute symptoms after menopause. Such adverse changes in physical and mental health may have an adverse effect on Quality Of Life (QOL) as they undergo transition
from menopause. The self-administered questionnaire that works well to classify women by their QOL and find out how their QOL benefits is known as Menopause-specific Quality of Life (MENQOL) questionnaire.

Pilates is a therapeutic exercise intended to enhance strength, stabilizer core muscles, endurance, muscle coordination, posture and respiration. Exercises can be done on mats or require the use of specialized equipment and the concepts of Pilates include centering that occurs by tightening of the body’s muscle core between the ribcage and the pelvic floor throughout exercise, focus where cognitive focus needed to accomplish the activity, balance that needs the posture and movement control throughout the activity, precision that allows the accurateness of the technique of exercise, flow to get smooth movement changes within the course of exercise and most importantly, respiration that needs proper inhalation and exhalation of air from the lungs in combination with the exercise. The Pilates mat training regimen results in young women’s physical and psychological health in terms of body weight, slimming, anxiety, depressed mood, tiredness and quality of life. Pilates helps to enhance both the mental and physical components in women during menopause.

Relaxation offers or helps to reduce anxiety, muscle tension and controls the pain. Progressive muscle relaxation technique (PMRT) is a therapy used since Edmond Jacobson’s implementation in 1938. PMRT involves tensing a muscle and then relaxing the tension; it is based on the psychological claim that people with stress and anxiety have tensed muscles and are able to relieve their pain both mentally and physically by learning how to relieve the tension. Music-accompanied PMRT can be a beneficial therapy to improve pain and QOL in pregnant women with LBP. Relaxation techniques lowers tension rates and enhance autonomic functions, cardiopulmonary performance and lipid profile. Researches had been conducted which shows positive effect of PMRT on cardiac patients, insomnia, chronic pain, anxiety. But there is paucity of evidence of PMRT effect in menopausal women on the QOL.

Sagdeo et al., 2011 conducted a study at Nagpur included 500 cases including 250 cases of rural and urban women stated that Indian scenarios should offer priority to menopausal health as menopausal women have improved life expectancy and population growth and presenting complaints which includes night sweats and hot flushes, heart distress, disturbances in sleep, depression, irritability, anxiousness, weight changes, physical and mental fatigue, joint and muscle pain, bladder problems, vaginal dryness.

Ghafari et al., carried out a review in the year 2009 on 33 diagnosed multiple sclerosis patient stated that PMRT is convenient and contributed to improving quality of life for multiple sclerosis patients.

Akbaş E et al., carried out a review in the year 2018 in which 26 participants were included in Pilates mat training stated that 6 weeks of Pilates training protocol leads to young women’s physical as well as psychological betterment as for the body fat, slimness, anxiousness, depressed mood, fatigue, and Quality Of Life.

Pilates is the physical training focuses on the breathing pattern which also helps in enhancing the QOL during menopause. The progressive muscle relaxation technique (PMRT) also helps to improve the QOL in several other conditions but not in menopausal women. It has been proven that PMRT reduces the stress in post-menopausal women. This intends to whether the PMRT increase the QOL of patient in menopausal women. The research is an initiative study that focuses on the effectiveness of PMRT on the various aspects of Quality Of Life of the menopausal women and comparison of the Pilates training and PMRT in order to enhance the QOL of participants.

**Objectives**

1. Pilates training effect in menopausal women on Quality Of Life
2. To find out the effect of Progressive Muscle Relaxation Technique in menopausal women in view of Quality Of Life
3. Comparison of Pilates training & Progressive Muscle Relaxation Technique on menopausal women’s Quality Of Life

**Methods**

This research is being conducted in local communities in Wardha City, Maharashtra, India after...
ethics clearance is obtained from Institutional Ethics Committee, Datta Meghe Institute of Medical Sciences, and Deemed to be University.

**Study design**: Comparative study

**Study setting**: Local communities from Wardha, Maharashtra, India.

**Participants**:

Inclusion criteria –
1. Any female in the age group more than 45 years
2. Participants willing to perform exercises
3. Menopause without any medical or surgical intervention
4. No contraindications related to performing the physical activity
5. Not indulged in any kind of structured physical activities or exercise program since 6 months

Exclusion criteria-
1. Menopause due to any surgical condition like oophorectomy, hysterectomy
2. Pathologies related to musculoskeletal and cardiovascular system
3. Cognitive impairments and neurological disorders
4. Recent Orthopedic surgery of lower back and lower limb
5. Hormone replacement therapy
6. Subjects on anti-depressant medications
7. History of vertigo or fall in previous 6 months

**Variables**

Outcome measures: Menopausal Quality Of Life (MENQOL)

**Data Source Measurement**-

After using intraclass correlation coefficients (ICC), the test-retest reliability were 0.81 for physical domain, 0.79 for psychosocial domain, and 0.70 for sexual domain, 0.37 for vasomotor domain and 0.55 for QOL. There are certain systematic changes demonstrated in vasomotor domain. The score of face validity was 4.7 out of 5. Validity of the evaluative construct suggested correlation coefficients in which physical domain was 0.60, vasomotor domain was 0.28, psychosocial domain was 0.55 and 0.54, and sexual domain was 0.54 and 0.32, and 0.12 for the QOL. Discriminative construct validity ranges from 0.57 to 0.70 in between all the domains.

**SAMPLE SIZE**: 42

**Intervention**:

**Group A**: Intervention will be Pilates Training Protocol.

**Group B**: Intervention will be Progressive Muscle Relaxation Technique

**Group A**:

A six week protocol has been made. The average length of every session would be 40 to 50 minutes including 5 minutes of warm up and cool-down each. The exercise prescription has been formed by the FITT (Frequency, Intensity, Time and Type of exercise) principle which includes frequency of 3 days a week for 6 weeks. Intensity has been calculated according to Rate of perceived exertion (RPE) scale and the progressions will be done with the same scale and there will be an interval of 10 seconds between each performed exercise. The exercises included in this intervention are considered as beginners and intermediate level exercise in order to fulfill the result. Therapist will demonstrate the activities by visual and verbal instructions to the patient and educate the patient about the correct form of exercise and how to perform it correctly. The main program consisted of exercise that illustrate 6 principles of Pilates includes- 1)Hundred 2) Half roll-down 3) Leg stretch (single and double) 4) Leg circle (single and double) 5) Rolling back (also known as rolling like a ball) 6) Spine stretch forward 7) Shoulder Bridge 8) Criss-cross and progression will be done accordingly.

**Group B**:

Division of body by four muscle group techniques occurs in such manner both upper limb and arms; the
face, neck & shoulder;\textsuperscript{3} the chest, back and abdomen; and\textsuperscript{4} both lower limb. It should be done alone, in a quiet place. Each group of muscles retains tension for 5 seconds and relaxes for 10 seconds.

The participant will be in a quiet place where there won’t be any interruption. They will lie down on back and stretch out in a comfortable manner. Guidance must be given to the participants to keep the muscle group tighter and tenser and to feel it. The participant will inhale deeply and tense first group of muscles for the given seconds. The tension should be in limit that they do not allow any cramps or pain of that muscle group. Relaxation of the muscle group along with exhalation will be done thereafter, which should be complete and sudden. Therapist will ask the participant to notice the differences in muscle group when they are tense and when they are relaxed.

The therapist will then help these muscles relax by advising them to remember what it feels like to relax those muscles in prior activities; this is the relaxation technique and recall. The process of counting involves count of 10 at the conclusion of the completed recall. This includes a profound technique of relaxation. The procedure is planned for 20 days, as well as a training program that could be done with 45 minute sessions.

Expected Results

Pilates training has been effective in Menopausal women for improving the Quality Of Life but Progressive Muscle Relaxation Technique has never been evaluated. The result would include the details about whether the measures are effective for improving the quality of life in menopausal women, as well as the comparison of both the interventions. Once the study result is complete, data will be analyzed using paired t-test and will be presented in the form of research paper.

Discussion

Menopause is defined as complete cessation of ovarian function by which the women experiences various symptoms after menopause which leads to changes in physical and mental health and may have an adverse effect on Quality Of Life (QOL). The aim of the study is to see the effect of both the interventions and compare the two interventions further i.e. Pilates Training versus Progressive Muscle Relaxation Technique on Quality Of Life in Menopausal women. Pilates is the physical training focuses on the breathing pattern which helps in enhancing the QOL during menopause. The progressive muscle relaxation technique (PMRT) helps to improve the QOL in several other conditions but not in menopausal women. In conclusion, this research seeks to explore both the effectiveness of Pilates and the Progressive Muscle Relaxation Technique in menopausal women. The result of the study would help prospective patients enhance quality of life. Outcome measure of the study is Menopause specific Quality Of Life (MENQOL) which is a self-administered questionnaire and it works well to classify women by their QOL and find out how their QOL benefits.

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Comparison of Muscle Length in Dominant Versus Non-Dominant Lower Extremity in Young Asymptomatic Individuals- A Research Protocol

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Abstract

Background: Muscle length is defined as the length at which the maximum amount of force a muscle is able to produce. This length is determined by the joint angle corresponding to that muscle. Understanding the optimal muscle length as well as its comparison between the extremities is very important as a part of examination in physiotherapy, particularly in the cases of musculoskeletal disorders. Several tests are available for testing the muscle length. However standardize and reliable tests have been chosen to prevent the error while testing. Many studies have shown that there was difference in the lengths of muscle of lower extremity which was assessed in different players. However there is paucity of study on the muscle length of individuals who are completely normal and not the athletes.

Objectives: The objective of this study is comparison between the length of iliopsoas, hamstring, gastrocnemius, rectus femoris in dominant to non-dominant extremity in young asymptomatic individuals aged between 18-25 years.

Methods: Measurement of hamstring, iliopsoas, rectus femoris and gastrocnemius length will be acquired through standard goniometer. The methods of assessment that will be used are; active knee extension (AKE) tests the hamstrings, Thomas and modified Thomas test to evaluate iliopsoas and rectus femoris while prone, figure-four position accompanied by dorsiflexion for gastrocnemius.

Results: Once the study is completed, the parameters of outcome measure will be statistically analyzed and calculated.

Conclusion: Based on the previous data we assume that there can be a significant difference between the muscle lengths of lower limb in normal person.

Keywords: Muscle length; Active knee extension (AKE) test; Modified Thomas test; Thomas test.

Introduction

One important feature of the skeletal muscle is the length tension relationship that reflects possible strength with relevancy the length of muscle. Important parameter during length-tension relation is optimal length of muscle, described as length wherein maximal force will be produced by muscle. This is determined by the joint angle comparable to the optimal muscle length.
This optimum angle shows the operating range in the length-tension relation through the movement of joint along with the excursion of tendon. So knowledge of various aspects of muscle contraction together with its mechanics and physiology is important since it plays a vital role during surgical procedures wherein the optimal length reflects how efficient surgical process could be, it helps in designing primary guidelines for ergonomic advice and in structuring a rehabilitation program that would help to provide more benefit using the advantages of length-tension relationship for an individual muscle.

Another concept namely muscle stress suggests of constant proportionality being balanced among maximum force of muscle and the physiologic cross sectional area. This parameter explains us the limitation in an individual muscle force.

It is common practice for physiotherapist to test length of muscle and flexibility as a section of assessment in patient with musculoskeletal disorders. Muscle length is simply measured by means of corresponding joint angle. Further length is determined on the basis of tests, comparison with normative values and between both the lower limbs. On the basis of this, exercises are been prescribed depending upon the information and other findings that is collected during examination time. Ranges that are obtained form an essential element during initial as well as recurrent clinic examination. Thus, identifying the length of muscle and their differences help us to know whether an individual would need any intervention focusing particular groups of muscle or joints. Also, the ranges obtained that of muscle length helps therapist to recognize individuals with reduced flexibility. This reduction in flexibility may be linked with an occurrence of painful event and injury to muscle of lower limb in future. To estimate the value of muscle length of extremity, it is necessary to make use of different techniques so as to get an objective measurement. Hence researchers have verified certain effectual techniques ensuring that the tests were easy to carry out, had higher inter and intra-tester reliability and they are clinical procedures usually practiced by the therapist.

There are several test available to measure the muscle length in lower extremity. Literature about muscle length testing had shown various techniques to test gastrocnemius length involving; passive range of motion, active range of motion, weight bearing techniques. Despite this, active dorsiflexion test is selected to check the length of gastrocnemius muscle because of its simplicity, reliability and standardization ease. A standard and reliable test that is chosen for the assessment of hamstring muscle length is active knee extension test (AKE) because its proven that it comprises of higher reliability that makes it a standardize test to use for an examination and stabilization of lower limb is achieved by this test, limiting the hip joint motion. Iliopsoas assessment to be done by the Thomas test while Modified Thomas test is chosen for testing the length of rectus femoris as one research have shown goniometer as a reliable tool to measure the hip extension using modified thomas test. It is a common and reliable test.

Parikh et al. 2015 conducted a study on establishing the typical values of muscle length of lower extremity, its comparison between dominant, non-dominant extremity of young elite cricket players having fifteen to twenty years of age. It has been found that decreased flexibility is common risk factor in cricketers and the players can even lose carrier due to an injury. On account of this, a study was done over 100 participants from one stadium. Because there was need to assess flexibility since it has huge impact on player’s performance and also it prevents musculoskeletal injuries, beside injury prevention is more desirable and better way for reducing injury in sports players. This research had helped the sports therapists to form specified muscle length norms. The result of study provided a reference range of muscle length in elite players. There was significant dissimilarity found between the lengths of rectus femoris, hamstrings, iliopsoas, and gastrocnemius of dominant and non-dominant side in these cricketers. Since the finding showed considerable difference between the dominant and non-dominant side in cricket players, it was suggesting the elite cricketers were more prone to the injury.

Marie corkery et al. 2007 done a study on establishing normative ranges for the lower extremities muscle length in seventy-two student of college. It aimed at obtaining the muscle length values in college students. Data was collected separately between males, females and a combined group. As per the result shown by the research a normative set of value was established for the lower extremity muscle length in resting state of
body. Implication of this study in clinical point of view incorporates individual’s examination plus diagnosis. Further they advised use of flexibility as a measure for an injury prediction. Moreover the defined normative data helped to determine flexibility and direct the strategies to resolve the deficits. However, lack of analysis of reliability before beginning with the study was found as a limitation of this work. Certain differences were assumed to be present within the range of measuring error. Less number of subjects as well as restricted age group is the second limitation of this study.

Although many research have been done incorporating muscle length, its normal value and comparison between the extremities, there is need for my study because there is paucity of study that evaluates muscle length in asymptomatic individuals and whether there is any difference in dominant to non-dominant side. Therefore this study will guide the therapist to know whether there is presence of difference in the muscle length of individuals that are normal.

**Objectives:**

To compare the length of gastrocnemius, iliopsoas, rectus femoris and hamstring muscle from dominant to non-dominant side of young asymptomatic individuals.

**Methods:**

**Study design:** observational study

**Setting:** Datta Meghe Institute of Medical Sciences (DMIMS), Sawangi (M), Wardha.

**Participants:**

Inclusion criteria:

- Individuals willing to participate
- Individuals between ages 18 to 25

Exclusion criteria:

- Previous history of surgery in lower extremity or low back.
- Any pathology or recent injury affecting lower limb and lower back (during current three months).
- Medicines or substance intake that would cause an altered sympathetic function.
- No elite athletes are accepted.

**Variables:**

**Outcome measures:**

Age, height, weight, gender, past medical history and surgical history will be recorded prior to the study with the use of questionnaire. Dominant leg will be obtained by asking the subject ‘what would be their preferred leg for kicking a football’. All four muscles will be measured initiating distally and moving proximally, right side before the left one. Both side lower limb muscle length measurement of hamstring, iliopsoas, rectus femoris and gastrocnemius muscle will be obtained using a standard goniometer. A goniometric measurement for the purpose of muscle length testing has shown to be reliable and also has better intrarater reliability than interrater reliability.

Gastrocnemius length will be measured in prone, figure-four position; foot to be measured hanged at the edge of the table. This position maintains the neutral attitude of lower extremity. While giving instruction, prior to starting a test, any trick movement performed by the subject will also be observed. The fulcrum of goniometer will be kept inferior to lateral malleolus, stationary arm parallel to fibula and movable arm lined with lateral aspect of calcaneum. Then subject will dorsiflex ankle and degree of dorsiflexion will be noted. There are many ways for testing the length of gastrocnemius however this position is chosen due to simplicity, less risk of researcher bias and excellent intra-rater reliability.

Active knee extension (AKE) test is chosen for assessment of length of hamstring. The subject will be taken into supine position with contralateral hip stabilize and knee flex to 90° as a starting point of reference. Fulcrum of goniometer will be placed laterally at knee, stationary arm parallel to femoral shaft while movable arm parallel to shaft of fibula. Subject is instructed to perform extension of knee unless a stretch will be experienced in the hamstring muscle and the knee angle will be measured where the subject felt some resistance very initially in the hamstring. A study was carried out for determining the reliability of active knee extension.
(AKE) test among healthy adults. Also some consider that Active knee extension (AKE) is a gold standard test for assessing hamstring flexibility. One research have demonstrated that the active knee extension test is easy to perform and require single person to handle with portable, simple, without any expensive apparatus and had also shown excellent interrater reliability and interclass correlation coefficient (ICC) values are 0.87 and 0.81, standard error of measurement (SEM) = 3.5 and 3.8 degree\textsuperscript{4}.

Assessment of rectus femoris length to be done by Modified Thomas test. The subject will stand at couch’s end with an instruction of holding the opposite knee and bringing towards own chest and further proceed towards supine lying with the one leg hanging outside table. Fulcrum of goniometer will be placing over lateral femoral condyle; stationary arm will be parallel laterally to femur and movable arm aligned with fibula in a line of lateral malleolus. This test will measure the range for knee flexion\textsuperscript{2}. A study on the examination of interrater reliability of goniometric measurement while assessing flexibility of hip extension, results of this study showed that goniometer was reliable instrument that can be used for measuring hip extension flexibility using modified thomas test. Interrater reliability (r=0.91-0.93), Interclass correlation coefficient (ICC) =0.89-0.92\textsuperscript{5}.

For assessing iliopsoas length Thomas Test will be used. The subject will be told to lie on table and the heels hanging out by the edge. The subject will then be instructed to pull leg towards chest to flattened lumbar spine on table. Fulcrum of goniometer will be placed over greater trochanter, stationary arm aligned with the midline of trunk, movable one parallel to lateral aspect of the thigh. This test measures the hip flexion angle. Thomas test has wide acceptance and common clinical tool to measure iliopsoas tightness\textsuperscript{2}. One study has been done about the reliability of thomas test while examining range of motion corresponding to hip. It showed Intrarater reliability=0.52 interrater reliability=0.60, Standard error of measurement (SEM) =1 degree\textsuperscript{6}.

**Study size:** 300

**Expected Results:**

Significant differences have been found in muscle length evaluated in different players. However, it has not been checked in individuals with no symptoms. Hence my study would help in knowing details about normal populations muscle length. Once it is studied and analysis is done, it is going to be presented as research paper.

**Discussion**

Optimum muscle length is the major factor to be considered in the length-tension relationship, whereas joint angle helps in determining the functional range by means of joint movement. Identification and knowledge of muscle length is very important as it has several beneficial role in an individual’s life including; need for an intervention strategies if any, while designing rehabilitation program for an individual, to evaluate pre-competition risk for injury in an athlete, testing the flexibility of muscle since decreased flexibility is commonly resulting into muscle injury. Many studies on the lower limb muscle length have been done in the past years. Such studies have given normative values as well as showed significant difference in an individual’s muscle length of lower extremity so far. But the study was done in a group of players and the result showed significant difference between muscle length of dominant and the non-dominant extremities of this athlete. Also normative set of muscle length values were found for the athletes. This is quite helpful for the sports therapist to know reference data about the players muscle length. However my study will be the first of its kind that would be assessing length of lower extremity muscles in the individuals who are completely asymptomatic. Individuals with past history of lower extremity disease or injury as well as the elite players will be prohibited. This is helpful as we will be able to obtain a data about muscle length in absolutely normal individual. Moreover this study will employ widely used methods for examination which are having appropriate reliability and validity as well as accepted by the researchers, found in literatures and research papers.

**Ethical Clearance:** Taken from institutional ethical committee.

**References**


Isoniazid Mono Resistance: Changing Trends in Drug Resistant Pulmonary Tuberculosis. – A Case Series of 3 Patients

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Abstract

Drug resistant tuberculosis is a major public health concern in many countries including India, Isoniazid resistance (INH) accounts for 7.2 % of new cases globally. In our case series 3 patients had INH mono resistance out of which 2 patients had no previous history anti tuberculosis therapy (ATT). All 3 patients received 6 months of standard regimen under National Tuberculosis Elimination Programme (NTEP). All patients showed clinical improvement. INH mono resistance diagnosis is often delayed due to lack of infrastructure of 1st line probe assay (LPA) at various centres. INH mono resistance if diagnosed early can also help in prevention of Multi drug resistant tuberculosis (MDR TB).

Keywords : INH mono resistance, LPA, MDR TB.

Introduction

Drug resistant tuberculosis is a major public health concern in many countries including India. In 2018 an estimated 3.4 % of new cases and 18 % of previously treated cases had MDR TB worldwide. India contributes to 27 % of MDR TB cases worldwide. The global average of INH resistance without concurrent rifampicin resistance is 7.2 % in new cases and 11.6 % in previously treated TB cases. However treatment is mainly focussed on MDR TB as all cases of rifampicin resistance are considered as MDR TB until proven otherwise. A TB patient can be labelled as INH mono resistance only after documented rifampicin susceptibility has been established. This can be achieved only through 1st line LPA or through culture drug susceptibility testing (DST). It is because of absence of rapid testing for INH resistance effective regimen is delayed and is associated with higher treatment failure.

Figure 1 : Chest x ray PA view showing bilateral upper zone cavities.

Case 1 : A 38 years old non immunocompromised male presented to us with complaints of dry cough, diffuse chest pain and significant weight loss for past 2 months. He had bilateral upper zone cavity on chest x ray posteroanterior (PA) view (figure 1). His induced...
sputum was 1+ for acid fast bacilli (AFB) on sputum microscopy and rifampicin resistance was not detected on cartridge based nucleic amplification test (CBNAAT). He was started on 4 drugs regimen consisting of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E). Patient came back after 3 weeks with worsening of symptoms and now presented with productive cough. His first line LPA was sent which showed INH mono resistance. Then he was started on 4 drugs consisting of R,Z,E and Levofloxacin (Lfx) for 6 months. At the end of 2 months his sputum converted to negative for AFB and showed significant clinical improvement.

Case 2 : A 50 years old non immunocompromised female presented to us with complaints of productive cough, loss of appetite and significant weight loss for past 1 month. She had history of pulmonary TB (PTB) 20 years back for which she was treated with ATT for 6 months. She had left upper zone cavitary lesion on chest x ray PA view (figure 2). Her sputum was 3+ positive for AFB on sputum microscopy. Her sputum sample was sent for CBNAAT and 1st line LPA. She was started on 4 drugs regimen consisting of H,R,Z,E. Her sputum CBNAAT showed no resistance for rifampicin but 1st line LPA showed INH mono resistance. Her treatment regimen was changed to R,Z,E and Lfx for 6 months. At the end of 2 months her sputum was negative for AFB and showed clinical improvement.

Case 3 : A 20 years old HIV negative female presented to us with complaints of diffuse chest pain, loss of appetite and productive cough for past 20 days. She was a known case of sickle cell disease. Her chest x ray PA view showed left lower zone cavitary lesion (figure 3). She was sputum positive for AFB on sputum microscopy and her sputum 1st line LPA showed INH mono resistance while sputum CBNNAT indicated no rifampicin resistance detected. She was started on 4 drugs regimen consisting of H,R,Z,E and was later changed to 6 months of R,Z,E and Lfx. She was sputum negative at the end of treatment and had clinical improvement.

Discussion

In the past INH mono resistance was detected through culture based DST and H resistance was determined at lower and higher concentrations. It is important because if H resistance is present at lower concentration and the TB bacilli is susceptible at higher concentration it can still be used to treat INH mono resistance. But this has not been established in clinical trials.\(^3\) 50 % to 95 % of INH resistant strains contain mutations in codon 315 WT1 of KatG gene and can be detected on 1st line LPA.\(^4\) KatG mutations are thought to cause high level INH resistance whereas low level resistance is caused by InhA mutations.\(^5\) In our case series we have presented 3 cases of INH mono resistance. 1st case diagnosis was delayed as he was thought to have drug sensitive TB based on his history and sputum microscopy. His diagnosis was
further delayed as sputum 1st line LPA is available only after 3 weeks at our institute. He showed significant improvement once he was started on latest INH mono resistance regimen under NTEP. In our 2nd case drug resistance was suspected as she had history of ATT intake in the past. She was started on drug susceptible anti TB drugs as we had no information regarding her resistance pattern and was deteriorating clinically. She was put on 4 drugs regimen for INH mono resistance. In our 3rd case also we ordered for 1st line sputum LPA based on our previous experience. She also improved clinically after being started on INH mono resistance regimen. In 2018 WHO released guidelines for treatment of INH mono resistance. It recommends 6 months of R,Z,E and Lfx in patients with INH resistance with confirmed rifampicin susceptibility. WHO recommends Lfx over moxifloxacin because of drug interaction with rifampicin.6 A 2017 systematic review and meta-analysis on treatment of INH resistant TB with 1st line ATT drugs suggests that such regimens can be suboptimal and lead to treatment failure, relapse or both.7

**Conclusion**

Primary INH mono resistance is on the rise as has been suggested in two of our cases and by their WHO global report 2019. All INH resistant patients must receive 4 drugs treatment regimen as suggested by WHO and NTEP for proper cure and improvement of patients. All INH resistant patients must be closely followed for development for MDR TB. Early diagnosis and treatment is key to management of INH mono resistance.

**Ethical Clearance**- Taken from SVIEC committee (letter attached)

**Source of Funding**- Non funded.

**Conflict of Interest** - Nil.

**References**

Study on Effectiveness of *Chakramarda* (*Cassia tora*) Ghrit and Go-Ghrit in the Management of Parikartika (Fissure in Ano)

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**Abstract**

The term Parikartika is used for the condition of Guda (anus) where cutting and burning pain occurs, also along with this pain occurs in anus, penis, umbilical region and neck of urinary bladder with cessation of flatus. In modern medicine trend of the management depends on the type of the disease, e.g. in cases of acute variety with short history of the problem can be treated on the conservative lines, which results in healing of almost all acute and majority of chronic fissures. Priority must be placed normalization of bowel habits such that the passage of stool causes less trauma to anoderm. The addition of fiber to the diet to bulk up the stool, stool softener and adequate water intake are the simple and helpful measures. In the present study, an effort was made to derive a standard and easily accessible treatment for fissure-in-ano. *Chakramarda* is having vraṇa shodhana andropaṇa properties which can help the Vraṇa (ulcer) to heal rapidly. Its base is Ghrit which itself is having Samskaranuvarti (i.e. it enhances the properties of drug) and healing properties. *Chakramarda* Ghrit is economically beneficial by virtue of easily available ingredients and a time tested classical formulation. Hence, it was selected for the clinical evaluation in the present study. It proved to be significant in managing Fissure in ano, it reduced pain, itching, bleeding and promoted healing. Properties of its chemical constituent’s probable mode of action can be derived that *Chakramarda* is efficient in healing any ulcer like anal fissure.

**Keywords:** Parikartika, Fissure-in-ano, Chakramarda, Ghrit, Samskaranuvarti

**Introduction**

In the age of fast food, there is a shift in the habit of taking food and its timings as well as in the lifestyle that has become sedentary. Both of these causes produce disturbance in the digestive system that leads to many diseases including anorectal disorders such as piles, fissure, fistula, prolapsed etc. constitute a significant category.

Anal Fissure is one of the major causes for pain at anal region. The fissure-in-ano is categorized into two types depending on the clinical symptoms & durations of the disease; viz. Acute and Chronic(1). The two primary signs of this disorder are, bleeding and pain; pain is often unbearable. In long-standing instances, sentinel tag and haemorrhoids can be associated with this. Pruritus ani can be another symptom of this disorder(2). In males anal fissure typically occurs in the midline posterior-90 percent and 10 percent much less frequently. Subsequently, female fissures on the anterior midline are somewhat more common than before. (60:40) (3).

In contemporary sciences Parikartika can be correlated with Fissure in Ano(4). Sources on Parikartika are available from all Bruhatrayi and corresponding writers of Ayurveda. Parikartika (fissure in Ano) is a very common condition. The factors responsible for the causation of Parikartika can be found in various Ayurvedic texts such as *Vamana – Virecana Vypada*, *Basti Karma vypada* and Upadrava of *Atisara, Grahani, Arsa*. In this regard, *Acharya Sushruta* stated the

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aetiopathogenesis of disease that if a anyone is impaired, with Mrudukoshta (mild digestive power), Mandagni (poor appetite) in these circumstances, more intake of food has the quality of Rukshna (dry), Ushna (hot), Lavana (salty) etc. Diet which is having such quality will vitiate Vata & Kapha & leads to Parikartika(5). The word Parikartika signifies Parikartanavatvedana around Guda i.e. cutting type of pain. Parikartika also has symptoms such as pain in the, penis, anus, neck of the urinary bladder and umbilical region with flatus cessation(6). In Kashyap Samhita, in the chapter Garbhini Chikitsa, we receive doshik classification, Aetiology, symptomatology and treatment of Parikartika(7). Acharya Charaka further mentioned fissure in ano as a Vataj Atisara complication(8). Chakramarda has Anti-inflammatory activity, Antibacterial, Antifungal Scavenging Activity, Antiulcer, Anti-proliferative activity, Antioxidant activity (9). In Ayurveda Chakramarda is used for the treatment of Pama, Jwara, Kasa, Kandu,Dadru. Rasapanchak of Chakramarda is Rasa-Katu,Guna-Laghu,Ushna, Virya-Ushan,Vipaka-Katu,Karma -Vata-Kaphasamak (10).

**Aim and Objectives**

The aim of the study was to evaluate the effectiveness of Chakramarda (cassia tora) Ghrit and Go-Ghrit in the management of Parikartika (Fissure in Ano). The defined objectives were to evaluate the efficacy of Go-Ghrit in the management of Parikartika as well as to the check and compare the efficacy of Chakramarda Ghrit and Go-Ghrit in the management of Parikartika.

**Material and Methods**

We have conducted a randomized single group blinded study with 30 patients in single group at outpatient and inpatient department of Shalyatantra department. The ethical approval was taken from Institutional ethics committee (Ref no.DMIMS(DU)/IEC/2017-18/7255 on 30/3/2017. We have included the patients from 18 years to 60 years with clinical features of Acute and Chronic Fissure in ano will be included after screening. The exclusion parameters were subject suffering with systemic disorders like Diabetes mellitus, Tuberculosis, HIV Positive, Hepatitis will be excluded as well as known cases of Malignancy, Crohn’s disease, Ulcerative colitis. We have also excluded chronic patient with 4th grade anal spasm. The diagnostic criterion was presence of signs and symptoms Parikartika such as Pain during defecation, Bright-red bleeding and Sentinal tag.

**Methodology**

The Ghrit was made by taking one part of kalka dravya (paste of seeds), four parts of cow ghee and sixteen parts of Drava (water) Table no.1. All the contents were mixed and prepared as per sneha pak vidhi. Leaves were collected from Wardha and nearby area. Ghrit was prepared in Rasa Shala of MGACH&RC by taking direction of subject expert. the dosage was Quantity sufficient for local application of Ghrit after Hot Sitz Bath twice a day during the treatment period. The total study duration was 45 days and assessment period was done on 0, 15th, 30th day and follow up of the patient was taken on 45th day.

3.2. Composition of Formulation:

<table>
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<tr>
<th>SR. NO</th>
<th>Ingredient</th>
<th>Botanical Name</th>
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<th>Proportion</th>
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<tr>
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<td>Casia tora</td>
<td>Seeds</td>
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<td>2.</td>
<td>Ghrit</td>
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<td>4part</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td></td>
<td></td>
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</tbody>
</table>

Investigations: For the research purpose we did Complete Blood Count, Random Blood Sugar.
**Subjective parameters:** The subjective parameters were Pain, Bleeding per Rectum, Itching, and Tenderness

**Objective parameters:** The objective parameter was Parikartika Healing

**Observations and Results**

Subjective and objective criteria were used to carry out the statistical analysis. Statistical analysis was done by using descriptive and inferential statistics using chi square test, Mann Whitney U test and Wilcoxon Signed Rank Test and software used in the analysis were SPSS 22.0 version and Graph Pad Prism 7.0 and p<0.05 is considered as level of significance. Changes before and after treatment are shown in figure 1, figure 2 and table no. 2.

**Table no2- Between the group comparison between baseline and after the treatment**

<table>
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<th>Symptoms</th>
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<th>SEM</th>
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<th>p-value</th>
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1. Pain

Comparison of pain in both the groups

Mean pain before treatment in patients of group A was 2.86±0.51 and in group B it was 2.73±0.45. By using Mann Whitney U Test statistically no significant difference was found in mean pain in patients of two groups (u=0.70, p-value=0.48).

Mean pain after treatment in patients of group A was 0.06±0.25 and in group B it was 1.40±0.63. By using Mann Whitney U Test statistically significant difference was found in mean pain in patients of two groups (u=4.55, p-value=0.0001). Percentage of relief in Pain of group A patients was 97.77% and in group B it was 49.99%.

2. Bleeding

Comparison of bleeding score in both the groups

Mean bleeding before treatment in patients of group A was 0.93±0.25 and in group B it was 1.00±0.00. By using Mann Whitney U Test statistically no significant difference was found in mean bleeding in patients of two groups (z=1.00, p value=0.31).

Mean bleeding after treatment in patients of group A was 0.00±0.00 and in group B it was 0.06±0.25. By using Mann Whitney U Test statistically significant difference was found in mean bleeding in patients of two groups (z=1.0055, p value=0.31). Percentage of relief in bleeding of group A patients was 100 % and in group B it was 80 %

3. Itching

Comparison of itching score in both the groups

Mean itching before treatment in patients of group A was 2.20±0.77 and in group B it was 2.33±0.81. By using Mann Whitney U Test statistically no significant difference was found in mean itching in patients of two groups (z=0.61, p-value=0.54). Mean itching after treatment in patients of group A was 0.46±0.50 and in group B it was 0.86±0.74 By using Mann Whitney U Test statistically significant difference was found in mean itching in patients of two groups (z=1.79,p-value=0.07). Percentage of relief in itching of group A patients was 86.66 % and in group B it was 67.77%.

4. Tenderness

Comparison of Tenderness score in both the groups

Mean Tenderness before treatment in patients of group A was 2.80±0.56 and in group B it was 3.00±0.00. By using Mann Whitney U Test statistically no significant difference was found in mean Tenderness in patients of two groups (z=1.44,p-value=0.15). Mean Tenderness after treatment in patients of group A was 0.13±0.35 and in group B it was 1.13±0.63. By using Mann Whitney U Test statistically significant difference was found in mean Tenderness in patients of two groups (z=3.95,p-value=0.001). Percentage of relief in Tenderness of group A patients was 95.55% and in group B it was 62.21

5. Fissure Healing

Comparison of Fissure Healing score in both the groups

Mean Fissure Healing before treatment in patients of group A was 2.80±0.41 and in group B it was 3.00±0.00. By using Mann Whitney U Test statistically no significant difference was found in mean Fissure Healing in patients of two groups(z=1.79,p-value=0.07). Mean Fissure Healing after treatment in patients of group A was 0.20±0.41 and in group B it was 1.20±0.67. By using Mann Whitney U Test statistically significant difference was found in mean Fissure Healing in patients of two groups(z=3.72,p-value=0.0001). Percentage of relief in fissure healing of group A patients was 95.55 % and in group B it was 59.99 %.

Discussion

1. Pain

When effect of Chakramarda Ghrit and Go- Ghrit on Pain was analyzed statistically by using Wilcoxon signed rank test the results were found significant in the patients of both the groups (on 15th, 30th and 45th day). On clinical assessment also Chakramarda Ghrit and Go-Ghrit, both the groups showed analgesic activity. On comparing the mean Pain in both the groups, by Mann Whitney U test statistically significant difference was found. Percentage of relief in Pain of Group A patients was 97.77 % and in Group B it was 49.99 %. Therefore it is clear from the above analysis that Chakramarda Ghrit was more efficient than Go -Ghrit in reducing pain
because of its healing property.

2 Bleeding

When effect of Chakramarda Ghrit and Go-Ghrit on bleeding was analyzed statistically by using Wilcoxon signed rank test the results were found significant in the patients of both the groups (on 15th, 30th and 45th day). On comparing the mean bleeding in both the groups, by Mann Whitney U test statistically significant difference was found (on 15th, 30th and 45th day). Percentage of relief in bleeding of group A patients was 100% and in group B it was 80%. Therefore it is clear from above discussion that Chakramarda Ghrit is more efficient than Go-Ghrit in stopping bleeding caused due to anal fissures which is also because of its wound healing property.

3 Itching

When effect of Chakramarda Ghrit and Go-Ghrit on itching was analyzed statistically by using Wilcoxon signed rank test the results were found significant in the patients of both the groups (on 15th, 30th and 45th day). On comparing the mean itching in both the groups, by Mann Whitney U test statistically significant difference was found (on 15th, 30th and 45th day). Percentage of relief in itching of group A patients was 86.66% and in group B it was 67.77%. From this we can conclude that Chakramarda Ghrit is more efficient than Go-Ghrit in reducing itching because of its antipruritic property.

4 Tenderness

When effect of Chakramarda Ghrit and Go-Ghrit on tenderness was analyzed statistically by using Wilcoxon signed rank test the results were found significant in the patients of both the groups (on 15th, 30th and 45th day). On comparing the mean tenderness in both the groups, by Mann Whitney U test statistically significant difference was found (on 15th, 30th and 45th day). Percentage of relief in tenderness of group A patients was 95.55% and in group B it was 62.21%. From the above discussion it can be concluded that Chakramarda Ghrit is more efficient than Go-Ghrit in reducing tenderness because of its anti-inflammatory and wound healing property.

5 Healing

When effect of Chakramarda Ghrit and Go-Ghrit on healing was analyzed statistically by using Wilcoxon signed rank test the results were found significant in the patients of both the groups (on 15th, 30th and 45th day). On comparing the mean healing in both the groups, by Mann Whitney U test statistically significant difference was found (on 15th, 30th and 45th day). Percentage of relief in healing of group A patients was 95.55% and in group B it was 59.99%. From the above discussion it can be concluded that Chakramarda Ghrit is more efficient than Go-Ghrit in healing anal fissure because of its wound healing property.

Taking into consideration all the observations, results, statistical analysis and its interpretation of the present study, Null hypothesis is rejected and Alternative hypothesis is accepted. Chakramarda Ghrit application in the management of Parikartika (Anal fissure) is found to be more effective as compared to Go Ghrit.

Conclusion

Chakramarda is a medicine used by people particularly for the management of skin disease and wound / ulcer. There have been numerous research works on the same where Chakramarda proved to be an effective medicine. Therefore using this comparison Chakramarda Ghrit was rendered and used for local application in the management of Anal fissure which is considered to be an ulcer, assessment parameters were itching, pain, bleeding, tenderness and healing on the basis of which its efficacy was assessed. The final conclusions were drawn on the basis of the Study observations and results described as follows

- Incidence of Fissure in ano was more common in middle age group.
- Males were more prone to this disease as compare to Females.
- Maximum cases were belonging to middle socioeconomic group, literate, married and having normal build.
- Fissure in ano was also very common in people who were indulged in alcohol, takes mixed diet and having irregular bowel habit.
- Maximum cases in this study belonged to rural habitat.
Majority of cases which reported were having acute fissure in ano following to that chronic fissure in ano with sentinel tag were common.

In this present research work, evaluation of the efficacy of Chakramarda Ghrit in the management of Parikartika (anal fissures) was done by comparing its results with that of Go-Ghrit. In this study, group A (n=15) was subjected to application of Chakramarda Ghrit and in group B, Go-Ghrit was used for application over Parikartika (anal fissures).

By this intervention the targets achieved in Group A (n=15), were that pain, itching, bleeding, tenderness were significantly reduced and healing was significantly more as compared to Group B (n=15) patients, where the Go-Ghrit was applied.

7. **Recommendations for further study**

- The study can be conducted in a substantial sample size.

- Chemical properties of Chakramarda Ghrit should be studied in more details.

- Chemical action of Chakramarda Ghrit in healing anal fissure should be studied.

- Chakramarda Ghrit preparation needs to be more hygienic and should be done with the use of specially designed device or equipment, even more easy method or technique should be find out for its application in order to make patient more comfortable to the treatment.

- Further same study can be conducted in comparison with other Ghrit preparation as well as modern medicaments with additional advanced changes.

**References**

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Percutaneous Subclavian Artery Covered Stent Placement Following Inadvertent Subclavian Arterial Cannulation

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Abstract

Inadvertent subclavian artery canulation although rare is a life threatening complication of central venous canulation. Its prompt recognition and management by stent placement, thrombin injection, Angio seal or gel foam embolization is essential to minimize fatal complications. We describe a case report of subclavian arterial canulation which was recognized and managed percutaneously by covered stent placement without complications.

Keywords: subclavian arterial; cannulation; covered stent

Introduction

Central venous catheter (CVC) cannulation in the subclavian vein of patients wounded in battle was first described by Aubaniac in 1952. [1] The CVC helps in the management of critical patients in that it provides reliable short to medium term venous access for hemodynamic monitoring, intravenous drug therapy, hemodialysis, parenteral nutrition and rapid fluid resuscitation and is now a commonly performed procedure in ICU setting. [2] Complications of Subclavian vein canulation include pneumothorax, hydrothorax, hemothorax, local hematoma, vascular injury, thrombo-embolism, and site related infections. [3] Inadvertent subclavian artery puncture during subclavian vein cannulation is a known and serious complication of CVC placement which could lead to hematoma, pseudo aneurysm formation, arterial occlusion, embolism, dissection, stroke, severe airway obstruction and even fatal bleeding on sheath removal as it is an anatomically non compressible site [4, 5]. Removal of inadvertently placed CVC followed by manual compression can lead to complications like uncontrolable bleeding and death. [6] Various techniques have been described to treat the subclavian artery after an inadvertent puncture like stent placement, thrombin injection, Angio seal and gel foam embolization. Techniques such as stent graft placement, thrombin injection, Angio seal devices and Gel foam embolization have been used to repair the subclavian artery after inadvertent puncture [7, 8]. We report a case of inadvertent subclavian artery cancellation, which was successfully closed using a covered stent placed percutaneously. A percutaneous approach is appropriate and justified management strategy with lesser complications as compared to surgical treatment. [9-11]

Case Report

A 45 -year-old gentleman, diagnosed with acute pancreatitis had a central venous catheter placement in the surgical ICU. As he was in shock an attempt was made to access the right subclavian vein to place the venous catheter by using an anatomic landmark technique with a 7F triple lumen central venous catheter. However, after gaining access the surgeons noted pulsating blood flow and an arterial puncture was suspected which was confirmed by sending the blood for arterial blood gas analysis. The CVC was not removed due to the risk...
of life-threatening bleeding which may occur and the inability to directly press the arterial leakage site.

The patient was taken to the cardiac Cath lab for an angiography. A bilateral femoral artery approach was made. A 6F JR4 catheter was inserted through the left femoral artery into the right subclavian artery and was used as a diagnostic catheter to inject the dye and visualize the site of subclavian arterial puncture. A 9F sheath was introduced into the right femoral artery and the stent was passed through it. The angiogram showed the entry of the CVC in his proximal portion of his right subclavian artery, after the right vertebral artery. (Fig 1 & 2)

We planned to pull out the catheter and cover the entry point at the Subclavian artery with a covered stent with due precaution to avoid occlusion of right vertebral artery. A Fluency® Plus Vascular Stent Graft (Bard, Inc.) 10 x 40 mm covered stent was positioned in the right subclavian artery (Fig 3), the misplaced venous catheter was removed (Fig 4 & 5). The position of the stent was reconfirmed by dye injection with respect to the site of entry of the catheter and origin of the vertebral artery and the stent was deployed (Fig6)

Immediately we achieved successful hemostasis and it was angiographically confirmed that the subclavian and vertebral artery was patent with no leakage of contrast from the puncture point (Fig 7) The patient was shifted to ICU and recovered and was discharged after 2nd day in a satisfactory condition and has been doing well on follow up. center mid-term results.
Discussion

There is extensive usage of a central venous catheter in critical care units to provide long term access and dedicated access for parenteral nutrition, hemodialysis and management of hydration in pre- and post-operative fasting states. Inadvertent arterial cannulation is seen in 3.2-3.7% of cases of CVC insertion. [12, 13] Usually a mistaken arterial puncture will be known by the spurt of blood from needle before insertion of a large bore canula, chances of arterial cannulation of CVC is very rare. [14] However patients with trauma or sepsis may be in shock and hence may not have pulsatile back flow and dark blood due to hypoxemic status and thus inadvertent arterial cannulation may not be recognized during needle puncture. Various other risk factors for complications in CVC has been described like obesity, short neck, urgent need for catheterization.[14] Arterial cannulation can be prevented by doing CVC cannulation under Ultrasound guidance. [15, 16]

Inadvertent arterial cannulations have been managed by removal of catheter and external compression, endovascular interventions like balloon inflation, stent placement, gel foam or Angio seal, or by surgical exploration and direct repair of arterial trauma. [6, 17, 18]. Inadvertently placed venous catheters have been traditionally removed directly with manual compression this practice is risky in arteries such as the subclavian artery, which are behind the clavicular bone and hence non compressible by direct pressure on skin. For removal of CVC by open surgical method the manubrium sterni, clavicle and first rib have to be resected in an extensive surgery. Direct pulling out of catheter and conservative monitoring for bleeding is not advisable currently because of increased mortality and complications with it and also availability of minimal invasive techniques these days [6, 19]. Endovascular approach is advisable for inadvertent arterial cannulations at sites which are difficult to compress manually or anatomically hidden areas. Subclavian vessels are large and readily accessible from the femoral or brachial approach making them well suited to endovascular therapy.[20] Technical success of endovascular repair in cases of subclavian artery injuries is reported between 94-100% with procedure-related complications between 0-22%. [21] Balloon tamponade has also been described as a accepted technique for endovascular repair of arterial punctures but carry a risk.
of dissection and failure needing a stent placement at the site. There are no randomized control trials or guidelines specifying advantage of one technique over other due to low incidence of such complications reported and broad clinical profile of patients with these complications. However percutaneous stent placement for inadvertent subclavian artery cannulation is a safe and easy procedure to prevent a life-threatening complication.

Conclusion

Inadvertent subclavian artery cannulation and hemorrhage can be a life-threatening complication during a commonly performed procedure. Ultrasound guidance and confirmation with non pulsatile back flow has been recommended as methods to avoid such complication. Manual compression, surgical repair and endovascular repair techniques have been described to handle such complications. We have described a case where inadvertent subclavian arterial cannulation was treated percutaneously with subclavian stent placement.

Ethical Clearance- As it is a case report consent from patient was taken for publishing article

Source of Funding- Self

Conflict of Interest- Nil

Bibliography


Correlation of Vitamin D level with severity of Coronary Artery Disease (CAD) in patients of Acute Coronary Syndrome (ACS) in a Tertiary Care Centre in Western India

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¹Associate Professor, ²DM Cardiology Senior Resident, ³Professor and HOD, Department of Cardiology, Smt. B. K. Shah Medical Institute and Research Centre, Sumandeeep Vidyapeeth Deemed to be University, Vadodara, Gujarat

Abstract

Background: Vitamin D deficiency (VDD) is a widespread problem in developing countries. Recent studies have shown association of Vitamin D deficiency with atherosclerosis and Coronary artery disease. This study was conducted to assess the relationship between Vitamin D and the severity of coronary artery disease (CAD) in patients undergoing coronary angiography.

Materials and Methods: Consecutive patients who underwent coronary angiography for acute coronary syndrome or effort angina were included in a descriptive cross-sectional study and their Vitamin D level was measured. CAD was defined as at least one vessel stenosis >50% on coronary angiography. Vitamin D levels were correlated to the CAG findings as to the severity and number of vessels involved and also to the traditional CAD risk factors.

Results: Vitamin D Deficiency was noted in 70.72% of the population in the study. Patients were classified as normal Vitamin D level (> 30ng/ml), Vitamin D insufficiency (20-30ng/ml) and Vitamin D Deficiency (<20ng/ml). Vitamin D levels had inversely correlated with significant coronary artery disease both for number of vessels involved and severity (p<0.001) and dyslipidemia (p=0.009). No significant association was found between VDD and other risk factors such as age, sex, hypertension, diabetes mellitus and smoking.

Conclusion: Vitamin D deficiency was noted in majority of the patients undergoing CAG and was found to be significantly associated with the prevalence and severity of CAD. However studies involving larger population are needed to evaluate whether supplementation with vitamin D may help in prevention of atherosclerosis and further development of CAD.

Keywords: Vitamin D, Coronary artery disease, Coronary angiography

Introduction

Presently Cardiovascular disease is the leading cause of mortality in India and this has reached epidemic proportions.[¹] In addition to the traditional risk factors of age, hypertension, diabetes mellitus etc, vitamin D Deficiency (VDD) is increasing being recognized to be associated with coronary artery disease and cardiovascular mortality and now considered as a risk factor for coronary atherosclerosis.[², ³] Also studies have shown that Vitamin D deficiency is associated with other cardiovascular comorbidites like diabetes mellitus, hypertension, dyslipidemia, obesity, peripheral vascular disease and heart failure.[⁴, ⁵] It has been shown that vitamin D deficiency affects vascular function,
accelerates atherosclerosis by plaque formation and progression and stimulation of systemic and vascular inflammation by upregulation of proinflammatory cytokines like TNF, IL-6 and upregulation of anti-inflammatory cytokine IL-10. Also Vitamin D has been found to have a role in inhibition of vascular calcification and its deficiency can lead to increased risk of calcified coronary atherosclerotic lesions.

Vitamin D is a fat soluble vitamin which acts like a prohormone and mediates its functions by binding to the nuclear receptor (Vitamin D Receptor). Vitamin D is synthesized in the skin as a pro-hormone on exposure to UltraViolet (UV-B) rays from sunlight as Ergocalciferol (Vitamin D2) and also absorbed in a small extent from the Gasterointestinal tract from food in form of Vitamin D3 (Cholecalciferol) and can be supplemented as Vitamin D3 in medication. All of these forms are activated in the liver to 25(OH)D, and further hydroxylated in the kidney to 1,25-dihydroxyvitamin D[1,25(OH)2D]. The 25(OH)D is a stable form not affected by level of calcium, phosphate and Parathyroid hormone and has a half-life of 2–3 weeks and hence can be used as measure of vitamin D level in blood. The optimal range is reported as 25-80 ng/ml while Vitamin D Insufficiency is reported as < 30 ng/ml and Vitamin D Deficiency (VDD) as < 20 ng/ml. The prevalence of vitamin D deficiency is higher in the developing countries with some studies showing upto 50% of population having VDD. Risk factors for development of Vit D deficiency are (i) decreased intake due reduced sun exposure and malnutrition (ii) decreased absorption due to celiac sprue, inflammatory bowel disease (iii) metabolic factors like liver and kidney disease. Depending on number of vessels involved they were classified into Normal coronaries, single vessel disease (SVD), double vessel disease (DVD) or Triple Vessel Disease (TVD). In addition to routine investigations all these patients underwent Vitamin D [25-(OH)D] level measurement in the Biochemistry Department by an autoanalyzer. A level of Vitamin D > 30ng/ml was considered as normal, 20-30 ng/ml as insufficient and < 20ng/ml as deficient.

Diabetes Mellitus, Hypertension, Dyslipidemia were defined based on standard definitions.

For quantitative variables under different groups undergoing CAG statistically analysis was done by using Mann-Whitney test. For qualitative variables chi square test was used. The data were analyzed using SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp and inference was drawn. A P value <0.05 was considered statistically significant.

Results

In our study of 263 patients who underwent CAG and Vitamin D level estimation, Vitamin D Deficiency was found in 186 (70.72%) patients, Vitamin D Insufficiency was found in 45 (17.11%) patients while normal Vitamin D level was found in 32 (12.16%) patients. There were 184 males (69.96%) and 79 females (30.03%) in the study population. Severe Vitamin D Deficiency (<20ng/ml) was found in 70.10% males and 72.15% females.

We conducted this study to determine the association of Vitamin D levels with severity of coronary artery disease in patients undergoing coronary angiogram and to establish correlation of Vitamin D Deficiency with conventional cardiac risk factors like Smoking, Hypertension, Dyslipidemia, Diabetes Mellitus and family history of CAD in patients.

Material and Methods

This was a cross sectional prospective study of 263 patients who underwent Coronary Angiography during a 3-month period at a tertiary care hospital in Western India. These included patients were of either gender and aged 18 to 75 years who underwent Coronary Angiography either for Acute Coronary Syndrome (Acute ST Elevation MI, Non-ST Elevation MI or Unstable Angina) or for Stable Effort Angina (symptomatic on medication). Patients who have liver or kidney disease, parathyroid disease, pregnancy, osteomalacia or taking drugs which affected Vitamin D metabolism were excluded from the study. Written and informed consent was taken from patients and their relatives for enrolment in the study.

Patients underwent CAG in the coronary Cath lab (PHILIPS FD-10) and interpretation of CAG was done by senior interventional cardiologist as to the number of vessels involved, severity of stenosis and calcification. The patients were divided into 3 groups based on the result of coronary angiography: Normal coronaries, non critical stenosis (<50% lumen stenosis of any coronary artery), and significant stenosis (>50% stenosis of a major coronary artery). Depending on number of vessels involved they were classified into Normal coronaries, single vessel disease (SVD), double vessel disease (DVD) or Triple Vessel Disease (TVD). In addition to routine investigations all these patients underwent Vitamin D [25-(OH)D] level measurement in the Biochemistry Department by an autoanalyzer. A level of Vitamin D > 30ng/ml was considered as normal, 20-30 ng/ml as insufficient and < 20ng/ml as deficient. Diabetes Mellitus, Hypertension, Dyslipidemia were defined based on standard definitions.

Material and Methods

This was a cross sectional prospective study of 263 patients who underwent Coronary Angiography during a 3-month period at a tertiary care hospital in Western India.
No statistical significant correlation was found between VDD and gender (p=0.732)

On evaluation of Vitamin D level with age, it was found that in the young (<30yrs) VDD was 50% while in > 30 years VDD was 74.43% and it was statistically significant (p=0.0018). (Table 1)

**Table 1: Correlation of Vitamin D status and age**

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<th>VitD &gt;30ng/ml</th>
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<tr>
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</table>

The level of Vitamin D was correlated with conventional risk factors for CAD like hypertension, Diabetes mellitus, Dyslipidemia and Smoking. 140 patients were hypertensive out of which 96 (68.57%) had VDD, while 90 (73%) of non hypertensives had VDD and this was not statistically significant (p=0.431). (Table 2) Similarly on evaluation of VDD with Diabetes mellitus, 163 patients were found to be diabetic, out of which 114 (69.93%) had VDD while 72 (72%) non diabetics had VDD. The difference was not statistically significant (p=0.72). (Table 3)

**Table 2: Correlation between Vitamin D status and hypertension**

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<th>Hypertension</th>
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**Table 3: Correlation between Vitamin D status and diabetes mellitus**

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<tr>
<td>Total</td>
<td>186</td>
<td>45</td>
<td>32</td>
<td>163</td>
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In our study 197 patients had dyslipidemia out of which 150 (76.14%) had VDD while 36 patients with normal lipid profile had VDD(54.54) and this correlation was found to be statistically significant (p=0.009)(Table4). Meanwhile there were 151 patients with history of smoking out of which 101 had VDD(51.41) while 85 non smokers(42.58) had VDD (p=0.11) which was statistically non significant
The correlation of CAG findings of CAD severity with respect to the Vitamin D level was also evaluated. Number of patients with normal coronaries were 16(6.08%), single vessel disease(SVD) was 81(30.74%), with double vessel disease(DVD) was 123(46.76%) and triple vessel disease was 43(16.43%). Out of patients with normal coronaries 2 patients (12.5%) had Vitamin D Deficiency, patients with SVD 57(70.37%) had VDD, patients with DVD 91(73.98%) had VDD while in patients with TVD 36(83.72%) had VDD. On statistical analysis it was found that for each level of severity of CAD it was inversely related to the level of Vit D and was statistically significant for patients with TVD(p<0.001). Patients with normal Vitamin D levels had less severe coronary artery disease compared to the ones with vitamin D level <20ng/ml. (Table 5). This is represented graphically with the graph showing the percentage of patients with CAD compared to the Vitamin D levels and shows the severity of CAD is inversely related to the level of Vitamin D in these patients (Figure 1)

Table 4: Correlation between Vitamin D status and dyslipidemia

<table>
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<tr>
<th>Dyslipidemia</th>
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</tbody>
</table>

Table 5: Correlation between Vitamin D status and Coronary Artery Disease

<table>
<thead>
<tr>
<th>No. of coronary vessels involved</th>
<th>Vit D &lt;20ng/ml</th>
<th>Vit D 20-30ng/ml</th>
<th>VitD &gt;30ng/ml</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (0)</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>SVD(1)</td>
<td>57</td>
<td>16</td>
<td>8</td>
<td>81</td>
</tr>
<tr>
<td>DVD(2)</td>
<td>91</td>
<td>17</td>
<td>15</td>
<td>123</td>
</tr>
<tr>
<td>TVD(3)</td>
<td>36</td>
<td>4</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>45</td>
<td>32</td>
<td>263</td>
</tr>
</tbody>
</table>
Discussion

There have been numerous studies in recent times highlighting the association of Vitamin D deficiency with higher incidence of CAD.[12-14] In fact these studies suggest that Vitamin D deficiency can act as an independent risk factor for CAD.[15, 16] Considering the limited data available from this part of India regrading Vitamin D level in patients with CAD this study was undertaken to correlate the risk of Vitamin D Deficiency with the severity of CAD along with their Coronary Angiogram findings.

In our study of 263 patients who underwent CAG, Vitamin D deficiency was detected in 70.72% patients. This number is similar to previous values in studies conducted in India.[17] The main finding of our study is a significant association between measured low Vitamin D level and the prevalence of severe CAD. Furthermore, as evident in figure 1 patients with Vitamin D Deficiency had significant coronary artery disease as compared to those who had normal Vitamin D level (p<0.001). This is in sync with studies by Chen et al, Danik et al etc have showed that low level of Vitamin D is associated with CAD.[13, 15, 18, 19]. Also angiographically it has been shown that the patients with low Vitamin D levels have higher number of coronary artery involvement. [16] In our study too VDD was present in more patients with SVD(70.37%), DVD(73.98) and TVD(83.72) and higher number of coronaries involved as compared to patients with normal Vitamin D levels. (Figure 1) Higher rate of Coronary Artery Calcification was also observed in our study indicating higher plaque burden in patients having VDD.

No significant difference between gender and Vitamin D level was noted in our study (p=0.732). Previous studies have however shown Vitamin D level to be lower in females compared to males [20] and this was attributed to females staying indoor, purdah and ghunghat practices, malnutrition and frequent pregnancies and lactation making them more Vitamin D deficient.

In our study we found Vitamin d deficiency more in the elderly compared to the younger population (p=0.018). This was also seen in previous studies where VDD was observed in elderly and postulated that with advancing age the ability of skin to synthesize Vitamin D decreases as well as they may have reduced renal and hepatic metabolism which affects the pathways of its synthesis. [10, 13]

In our study we found no significant correlation of Vitamin D levels with relation to Hypertension(p=0.431) and patients with Diabetes (p= 0.72). Some studies have shown that Vitamin D level are lower in patients with
hypertension\cite{13, 19, 21} and Diabetes \cite{19, 22, 23}. Smoking inhibits Vitamin D induced translocation of VDR from the nucleus to the cell membrane, VDD has been observed in previous studies in smokers\cite{24} our study found no significant correlation between smoking and VDD(p=0.11). We found dyslipidemia to be significantly correlated with Vitamin D deficiency(p=0.009). This has been observed in previous studies by Rolf et al, \cite{25} Vitamin D deficiency affecting calcium absorption, Triglyceride metabolism, insulin resistance, disruption of lipoprotein metabolism leading to dyslipidemia have been postulated\cite{26}

**Limitations**

This study was conducted on 263 patients and findings should be confirmed in large sample studies before being generalized. This was an observational study and the association of Vitamin D with CAD can’t be postulated as a causal factor for the same and needs experimental and randomized control trials to evaluate this hypothesis further. This was a hospital-based cohort study from a single center and not a population based study and findings cannot be generalized for the entire population

**Conclusion**

The field of interventional cardiology has progressed at a fast rate with newer techniques of stenting techniques and pharmacological advances with dual antiplatelets and statins but still progression of atherosclerosis remains uncontrolled especially in high risk patients. Though conventional risk factors like Diabetes, Hypertension, smoking, dyslipidemia are given importance in the workup of CAD, new risk factors like low vitamin D level are not being recognized and also it can be corrected much easily compared to other traditional risk factors. Our study shows VDD is widespread in India and also has a significant correlation to CAD. More widespread studies involving multiple centres and more patients should be undertaken to study the association further and steps for correction of Vitamin D deficiency in the population should be undertaken.

**Ethical Clearance**- Observational study and no intervention done in human subjects - institutional review committee was informed and study cleared.

**Source of Funding**- Self

**Conflict of Interest** – Nil

**Bibliography**


A Questionnaire-Based Study to Evaluate the Basic Understanding of Pharmacovigilance of the Under Graduate Medical Students of a Rural Teaching Hospital

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Abstract

Introduction: Adverse Drug Reactions (ADRs) have presently been greatly contributing to the hospital admissions, prolongation of the hospital stays, visits to the emergency departments, in turn, contributing to the economic burden of healthcare management. Pharmacovigilance is predominantly concerned with ADRs and drug safety. The basic understanding about pharmacovigilance is essential for future medical professionals as they will come in contact with the patients and can efficiently report the ADRs.

Aim: To evaluate the basic understanding about pharmacovigilance and create awareness in medical students of a rural teaching hospital.

Materials & Methods: This was a non-interventional questionnaire-based study, where medical students from a rural teaching hospital were included in the study. They were distributed a simple questionnaire related to pharmacovigilance basic knowledge, through google form link, which they had submitted. The data was analysed.

Results: Total 92 participants were included in the study. It was found that, 57.8% of these were female and 42.2% were males. Moreover, regarding assessing the basic pharmacovigilance knowledge it was reported that 98.9% were aware about healthcare professionals who can report ADR. Nearly 83.5% were aware about pharmacovigilance. Nearly 70% of the participants had not heard about pharmacovigilance before joining pharmacology lectures. Majority of the participants had never attended any seminar/workshops and agreed to attend in future.

Conclusion: The results indicate that the basic understanding about pharmacovigilance is improving and medical student shows interest about creating awareness related to pharmacovigilance, as it is an important aspect of drug safety. However, to further improve their understanding awareness programs for medical student can be conducted.

Keywords: Adverse Drug Reactions, CDSCO, MBBS students, Medical under graduates, Pharmacovigilance, WHO

Introduction

Paracelsus stated that, all substances (drugs) are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy¹. The safety and efficacy of the drugs used in the treatment of various clinical conditions in any individual remains complex and multifactorial and difficult to analyse or identify the suspected drug that causes the Adverse Drug Reaction
ADR). Adverse effects usually predict hazard from future administration and warrant prevention, or specific treatment, or alteration of the dosage regimen, or withdrawal of the product. Continuous monitoring of drug effects, side effects, contraindications and outright harmful effects which could result in a high degree of morbidity, and in some cases, even mortality, are essential to maximize benefits and minimize risks. As mentioned by WHO Collaborating Centre for International Drug Monitoring, Uppsala, Sweden WHO promotes pharmacovigilance at the country level. The Central Drugs Standard Control Organisation (CDSCO), New Delhi, has started a National Pharmacovigilance Programme.

Therefore, looking to the above facts, it is important to safeguard patient safety and must be applied at all healthcare establishments. However, deficiency of awareness, training, and underreporting of Adverse Drug Reactions (ADRs) are the major difficulties in the successful application of Pharmacovigilance (PV) programmes.

Adverse drug reactions (ADRs) have presently been greatly contributing to the hospital admissions, prolongation of the hospital stay, visits to the emergency departments, in turn, contributing to the economic burden of healthcare management. Studies have shown that 4.2% - 30% of the hospital admissions, in U.S.A.; occur due to the ADRs. The ADRs that occur, affect the individuals irrespective of their age, gender, weight, race, or ethnicity. This is evident with several epidemiological studies conducted across the world which show that 2.1% - 5.2% of the ADRs have been observed to occur in children contributing to their hospital admissions, of which 39% of the ADRs were considered to be life-threatening or fatal while 10-20% of the ADRs occurred among the geriatric patients, while 11.4% - 35.5% of the visit to emergency department have been related to the ADRs. It has also been observed that 32%-65% of the ADRs were found occurred in geriatric cases admitted to the nursing homes. Thus, the ADRs greatly contribute to the economic burden in healthcare management as the treatment of ADRs is more costly than treatment of the diseased condition. Although, the investigation of the expenditures on the adverse drug events (ADEs) is less, the implications of research makes it clear that the ADRs cause injury to the patients with a disproportionate increase in the expenses to treat these injuries. Moreover, for certain conditions or disease either for the prevention nor for the treatment, there have been no drugs or vaccines proven to be effective and molecules are being investigated and developed as potential therapies or as adjuvant therapies therefore there is need for pharmacovigilance.

Therefore, it is known that pharmacovigilance is predominantly concerned with ADRs, and drug safety. The basic understanding about pharmacovigilance is essential for future medical professionals as they will come in contact with the patients and can efficiently report the ADRs. Hence, the present study was aimed to evaluate the basic understanding about pharmacovigilance and create awareness in medical students of a rural teaching hospital.

**Materials & Methods**

The present observational (non-interventional) questionnaire-based study was conducted over a period of one month at S. B. K. S. Med. Inst. & Res. Centre, Sumandeep Vidyapeeth An institution deemed-to be University; the study was conducted amongst MBBS students. The required permission was obtained. The required consent for participation for the study was obtained from the under graduate students of MBBS. The participants were 92 undergraduate medical students of S. B. K. S. Med. Inst. & Res. Centre, Sumandeep Vidyapeeth An institution deemed-to be University; they were enrolled in the study. A total of 92 questionnaires were shared through google form link (total n=92) which included total 20 questions based on earlier study and other sources. The questionnaires were based on answering from options or as short answers related to the pharmacovigilance basic understanding amongst undergraduate medical (MBBS) students. Data collected was further entered in Microsoft excel sheet and were further analysed.

**Inclusion criteria:** Medical undergraduates from S. B. K. S. Med. Inst. & Res. Centre were included in the study.

Those participants who gave consent.

**Exclusion criteria:** Students not willing to participate in the study or not willing to give consent.
Results and Discussion

The results were evaluated based on the detail questionnaire and its analysis done. The results were grouped as medical students’ knowledge, and basic understanding on pharmacovigilance as shown in Table no 1.

Among the total of 92 participants (n=92) who participated in this present research study; 57.8% of these were female and 42.2% were males. The significant outcome was, 98.9% were aware about healthcare professionals who can report ADR. 83.5% were aware about pharmacovigilance. Majority of them, 96.7% had heard about clinical trials earlier, however only 69% knew about pharmacovigilance in which phase of clinical trial. Almost all, 98.9% knew about suspected ADR reporting form. 94.5 % have heard about PvPI. Nearly 70% of the participants had not heard about pharmacovigilance before joining pharmacology lectures. Majority of the participants had never attended any seminar/workshops and agreed to attend in future. Also, nearly all MBBS students 96.7% were aware about the terminology ADR. The details are listed in table no 1.

Table no. 1: Questionnaire to access UG students’ knowledge regarding pharmacovigilance basic understanding (n=92):

<table>
<thead>
<tr>
<th>Q. no.</th>
<th>Questionnaire2,5,6,7 (in brief)</th>
<th>Response (Yes/No) from participants n=92 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q. 1</td>
<td>Healthcare professional includes dentists, physicians &amp; pharmacists.</td>
<td>98.9 (Yes)</td>
</tr>
<tr>
<td>Q. 2</td>
<td>Pharmacovigilance is about drug safety.</td>
<td>83.5 (Yes)</td>
</tr>
<tr>
<td>Q. 3</td>
<td>Heard about “clinical trials” earlier.</td>
<td>96.7 (Yes)</td>
</tr>
<tr>
<td>Q. 4</td>
<td>Pharmacovigilance comes under Fourth phase of clinical trials.</td>
<td>69 (Yes)</td>
</tr>
<tr>
<td>Q. 5</td>
<td>Aware about Suspected ADR reporting form/white form.</td>
<td>98.9 (Yes)</td>
</tr>
<tr>
<td>Q. 6</td>
<td>Knows about of pharmacovigilance programme in India.</td>
<td>94.5 (Yes)</td>
</tr>
<tr>
<td>Q. 7</td>
<td>Agree that ADRs might result in hospital admission, prolonged hospitalization or might lead to permanent disability or even death.</td>
<td>91.1 (Yes)</td>
</tr>
<tr>
<td>Q. 8</td>
<td>Knows the terminology ADR.</td>
<td>96.7 (Yes)</td>
</tr>
<tr>
<td>Q. 9</td>
<td>Know about which kind of ADR’s need to be reported.</td>
<td>89 (Yes)</td>
</tr>
<tr>
<td>Q. 10</td>
<td>Had NOT heard about terminology “Pharmacovigilance” before joining Pharmacology Lectures.</td>
<td>70 (Reply No)</td>
</tr>
<tr>
<td>Q. 11</td>
<td>Knows the full form of ADR.</td>
<td>89 (Yes)</td>
</tr>
<tr>
<td>Q. 12</td>
<td>Knows that adverse event and adverse effect are different and knows the difference.</td>
<td>69 (Yes)</td>
</tr>
<tr>
<td>Q. 13</td>
<td>Agreed that they have ever filled any ADR form. During Pharmacology practical.</td>
<td>86 (Yes)</td>
</tr>
<tr>
<td>Q. 14</td>
<td>Will motivate any of the healthcare providers to fill ADR form.</td>
<td>79 (Yes)</td>
</tr>
<tr>
<td>Q. 15</td>
<td>Aware of any ADR monitoring centre in our institution where you can submit the filled ADR form and its location.</td>
<td>76 (Yes)</td>
</tr>
<tr>
<td>Q. 16</td>
<td>Gave any suggestions to improve ADR reporting.</td>
<td>55 (Yes)</td>
</tr>
<tr>
<td>Q. 17</td>
<td>They had NEVER attended any workshop/seminar/training programme related to pharmacovigilance.</td>
<td>77.3 (Reply No)</td>
</tr>
<tr>
<td>Q. 18</td>
<td>Interested in attending any seminar/training programme related to pharmacovigilance</td>
<td>31.1 (Yes)</td>
</tr>
<tr>
<td>Q. 19</td>
<td>Knows different Types of ADR.</td>
<td>72 (Yes)</td>
</tr>
<tr>
<td>Q. 20</td>
<td>Knows about PvPI.</td>
<td>70 (Yes)</td>
</tr>
</tbody>
</table>

Out of total 92 participants, 90 number of participants had responded which is depicted in below mentioned Table no. 2. Gender ration was calculated, which shows the percentage distribution of male and female participants in Table no. 2.
Table no. 2: Assessment of gender ratio of the participants:

<table>
<thead>
<tr>
<th>Gender Ratio (n= 90)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Participants =</td>
<td>52</td>
</tr>
<tr>
<td>Male Participants =</td>
<td>38</td>
</tr>
<tr>
<td>Total MBBS UG students who responded</td>
<td>90</td>
</tr>
</tbody>
</table>

The detailed distribution of each question is depicted in Table no. 1 and some significant distribution is depicted in Figure no. 1 (a -d) as mentioned below.

**Figure no. 1: Distribution of percentage to access UG students’ knowledge based on questionnaire regarding pharmacovigilance basic understanding:**

(a) Pharmacovigilance comes under Fourth phase of clinical trials.

Q. 4 Pharmacovigilance comes under which phase of clinical trials?
60 / 87 correct responses

![Pharmacovigilance phase distribution chart](chart.png)

(b) Knows about of pharmacovigilance programme in India.

Q. 6 Are you aware of pharmacovigilance programme in India?
91 responses

![Pharmacovigilance awareness chart](chart.png)
(c) Agree that ADRs might result in hospital admission, prolonged hospitalization or might lead to permanent disability or even death.

Q. 7 Do you agree ADRs might result in hospital admission, prolonged hospitalization or might lead to permanent disability or even death?
90 responses

Yes: 91.1%
No: 30%
Not sure: 70%

(d) Had heard about terminology “Pharmacovigilance” before joining Pharmacology Lectures.

Q. 10 Had you heard about terminology "Pharmacovigilance" before joining Pharmacology Lectures?
90 responses

Yes: 70%
No: 30%

The present study provides an important insight regarding the knowledge, about basic understanding on pharmacovigilance undergraduate future clinicians. Almost all the MBBS students have the basic knowledge, however, it is very essential to sensitize the undergraduate students on the importance of Pharmacovigilance program of India, and also regarding Post marketing surveillance Phase.

Hence, this will help us to learn the design and will help us to narrow down the distance between academic knowledge among future clinicians and clinical practice. Majority of the students have given suggestions so as to improve ADR reporting and though majority of them have not attended and seminar/workshop, they are interested in attending such programs.

Conclusion

The results indicate that the basic understanding about pharmacovigilance is improving amongst MBBS students. Also, medical students show interest about creating awareness related to pharmacovigilance, as it is an important aspect of drug safety. However, to further improve their understanding and to sensitize them, awareness programs for medical student can be conducted.
Acknowledgments: Author is grateful to Smt. Bhikhiben Kanjibhai Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth an Institution deemed to be University, Piparia, for permitting to conduct the present study.

Research Funding: No funding involved.

Competing Interests: NIL.

Ethical Clearance: As the present study is based on google form link questionnaire online method and does not involve any risk to humans, the consent from the participants is taken and their identity is not disclosed.

References
Assessing Fear-Avoidance Belief Questionnaire and Quality of Life in Housewives with Knee Osteoarthritis: A Research Protocol

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Abstract

Introduction - Osteoarthritis (OA) is an inflammatory disorder of cartilage, degeneration of synovial fluid, osteophyte formation, thinning of joint space and sub-chondral sclerosis. The prevalence of osteoarthritis in India was found to be around 28.7%. Females are generally more affected than males with the ratio of 2:1. Of all the joints knee and hip joint are seen to be most commonly affected joint in OA. Prevalence of knee pain is common amongst housewives due to prolong standing and frequent bending. Strenuous physical activity, especially activities requiring kneeling, knee-bending, stair climbing, squatting, prolonged standing as well as knee injury and trauma have also been linked to a high prevalence of symptomatic knee OA. Knee OA causes pain of various degree depending upon the severity of disease which can cause fear in patients. The fear related to pain can result in decrease in quality of life.

Purpose - Purpose of this study was to assess fear amongst housewives related to OA using fear-avoidance belief questionnaire and the effects of fear on their daily life using WHOQOL-BREF.

Methodology - 200 housewives will be selected according to exclusion and inclusion criteria. Their knee radiography was done to look for level of OA. Then those ladies were interview to find their fear related to disease and its effects and how that affects their quality of life.

Results - Results are awaited till the study is completed.

Conclusion - Conclusion of this study depends upon the result of this study.

Keywords – avoidance; fear; housewives; inflammation; knee pain; osteoarthritis; quality of life;

Introduction

Osteoarthritis (OA) is an inflammatory disorder of cartilage, degeneration of synovial fluid, osteophyte formation, thinning of joint space and sub-chondral sclerosis(1)(2). The radiographic evidence worldwide reflects 25.4% prevalence while 15.4% being symptomatic or clinically defined knee OA worldwide(3). The prevalence of osteoarthritis in India was found to be around 28.7% (4). The prevalence of OA in Maharashtra is 10.2% , in females (11%) and males (7%) (5). Osteoarthritis is more common in female than male, its ratio is approximately 2:1 (6). OA is now recognized as a disease involving the entire joint including periaricular musculature(7). Therefore accordingly, the impairment,
activity limitation, and participation restriction related to OA goes far beyond the radar of synovial joint. OA leads to pain, disability as well as difficulty in joints and restrict the routine movements of human being. No single factor predisposing an individual to OA has been identified, although aging is indeed strongly associated with OA. Genetic factor account for between 39% to 65% of radiographic OA of hand, hip, knee and upto 70% in spinal OA. Of all the joints knee and hip joint are seen to be most commonly affected joint in OA. Strenuous physical activity, especially activities requiring kneeling, knee-bending, stair climbing, squatting, prolonged standing as well as knee injury and trauma have also been linked to a high prevalence of symptomatic knee OA. Malalignment, including varus and valgus deformities, and leg length discrepancy are associated with greater prevalence of knee and hip OA, respectively. Obesity has also shown risk factor for development of knee OA in later life.

OA typically starts insidiously and gradually progresses and may even go unnoticed in some individuals when there is involvement of aneural articular cartilage. Pain is initial symptom. In later stages pain becomes dull aching, chronic and accentuated with episodic severe pain. There can be presence of synovial thickness as indicated by a joint tenderness with effusion, crepitation are felt during joint movement. OA is generally classified by Kellgren and Lawrence’s classification which is based on osteophytes formation using five grades from 0 to 4.

Knee OA more commonly affects the medical joint due to greater load body-weight placed on this compartment. As a result, medial joint space narrowing occurs resulting in pseudo laxity of medial collateral ligament, stretching of lateral collateral counterpart, and a genu varus deformity. Genu valgus occurs due to greater lateral compartment involvement but is less common. A flexion deformity of several degrees can develop quickly in painful knee and contribute to functional leg length discrepancy, decrease step length and quadriceps muscular fatigue or strain. Patellofemoral compartment OA, with hallmark anterior knee pain, can occur in isolation as a result of patellar malalignment, abnormal tracking and loading, and direct trauma to patella.

Vast pharmacological and non-pharmacological treatment protocols are available for osteoarthritis depending upon the severity of the disease. Medical therapies focus on decreasing pain and inflammation. Early aggressive pharmacological therapy is associated with diminished joint damage and long-term maintenance of function. The physical therapy plays a pivotal role in helping patient with minimal disability gain confidence and experience in using self-management skills to deal with the condition.

In Indians, primary OA of knee is more common than secondary OA. It is perhaps due to frequent need of squatting and sitting cross-legged. Prevalence of knee pain is common amongst housewives due to prolong standing and frequent bending. The fear related to pain is seen amongst people. This gives rise in decrease in quality of life.

The World Health Organization Quality of Life Instrument, Short Form (WHOQOL-BREF) questionnaire is a commonly utilized generic measure of quality of life that is used to measure quality of life in healthy people and in different groups of patients. It is a short version of WHOQOL-100 scale which is used in larger research group and clinical trials. WHOQOL-Bref comprises of four main domains which is physical health, psychological, social relationship and, environment. It contains of total 26 item questionnaire that correlates well with the original WHOQOL-100 item questionnaire. It assess the individual’s perception in the context of his/her cultural and value system, personal goal, standards, concerns, satisfaction with life and many more. Of the 26 items, 24 items were used to calculate the four QOL domains: physical health (7 items), psychological (6 items), social relationships (3 items) and environment (8 items). Transformed domain scores range from 4 to 20. A higher score indicates a better QOL. The two remaining items, sometimes used to calculate overall QOL and health, were not used in this study as recommended by the WHO. The WHOQOL-Bref have been shown to display good discrimination validity, content validity and test-retest reliability.

Fear avoidance beliefs are measured by using validated self-reported questionnaire which is created by Waddle et al, the Fear avoidance belief questionnaire. The Fear-Avoidance Beliefs
Questionnaire (FABQ) is a 16-item self-report questionnaire that focuses particularly on patient’s fear avoidance beliefs about physical activity and work may affect and contribute to their pain and concomitant disability. Fields of assessment involves; Activities of Daily Living, behaviour, functional mobility, general health, life participation, mental health, motivation, occupational performance, pain, personality, QOL, self-efficacy, stress and coping(15). The theory of fear avoidance is based on the notion that the patient believes incorrectly that the pain is sign of harm(16). Fear of pain leads to avoidance behaviour, such as rest and consequent avoiding of physical activity can result in worsening of patient’s condition(16). It has been proposed that fear-avoidance behaviours are expressions of a person’s belief system and that alteration of this belief system can cause in a more productive concept of pain and thus more positive behavioural responses, such as a steady return to activity and work(16). This self-reported questionnaire contains 16 questions ranging from 0 to 6 (maximum score of 96; higher score indicates fear avoidance behaviour). The first 5 questions concern to physical activity while the remaining 11 discuss about work. The Physical Activity subscale (FABQ-PA, range from 0 to 24) is the total of component from 2 to 5. The Work subscale (FABQ-W, range from 0 to 42) is the total of component 6,7,9-12 and 15. The overall test-retest reliability of FABQ was found to be good (ICC=0.97)(17). Evidence shows correlation between FABQ and Roland & Morris Disability Questionnaire. The correlation co-efficient for the FABQ was 0.52, the FABQ Work subscale was 0.63 and the FABQ Physical Activity subscale was 0.51(17). The reliability of forty-eight hour test-retest for FABQ has been recorded with a Pearson r of 0.91 to 0.95 and 0.84 to 0.88 for FABQ physical activity scale(14).

Gordon Waddel et al,(1993) conducted a study on FABQ and the role of fear-avoidance beliefs in chronic low back pain and disability in which he reasoned that the importance of fear-avoidance beliefs and showed that specific fear-avoidance beliefs about work are closely related to loss of work because of low back pain(14). Asa Dedering et al, (2013) conducted a study on assessing Fear-avoidance Beliefs in patients with cervical radiculopathy in which he deducted that FABQ can be used for test–retest evaluations as ‘good’ reliability was found. Marcio Massao Kawano et al, (2015) conducted a study on assessing QOL in patients with knee OA in which he interpreted that individuals with OA have a low perception of their functional capacity, functional limitation and pain in QOL. There is a clear link between poor education and low perception of quality of life(18). Rajeetha Miraj et al,(2018) conducted a study on prevalence of knee osteoarthritis in women of rural and urban part of Jaffan in which she reasoned that osteoarthritis was higher in urban areas (42%) than rural areas (16%) (19).

Chirstine Becks Mansfield et al, (2018), conducted a study on the effects of fear-avoidance beliefs on anterior knee pain and physical therapy visit count for young individuals in which he conclude that Fear-avoidance beliefs in adolescents are similar to that seen in adults. FABQ-PA scores did not impact the number of visits used to treat anterior knee pain or functional ability at discharge(20). Sara R. Piva et al, (2009), conducted a study on predictors of pain and function outcome after rehabilitation in patients with patellofemoral pain syndrome in which she concluded that change in fear-avoidance beliefs about physical activity was the strongest predictor of function and pain outcome. The fact that patients who decreased their fear-avoidance beliefs improved function and decreased pain indicates that perhaps fear-avoidance beliefs should be targeted during the treatment of patients with patellofemoral pain syndrome(21). Jack Farr II et al, (2013), conducted a study on quality of life in patients with knee osteoarthritis: A commentary on nonsurgical and surgical treatments in which he concluded that surgical knee OA intervention generally results in good to excellent patient outcome. Knee OA has negative impact on health related quality of life (HRQoL)(22). Fatma Fidan et al, (2012), conducted a study on quality of life and self-reported disability in patients with knee osteoarthritis in which she concluded that patients with knee OA has significant poorer Qol compared with healthy controls. SF-36 is related to the clinical status and functional ability of patients with OA and can be used as sensitive health status measure for clinical evaluation. Also WOMAC can be used as a sensitive measure for disability of patients with knee OA(23).

In this study we hypothesize that due to fear of increasing pain due to movement there was deterioration in quality of life especially in level of independence.
Greater the pain and severity of OA larger will be the deterioration in quality of life.

In this study we are assessing Indian housewives with knee pain/Osteoarthritis with Fear-avoidance Belief Questionnaire and Quality of Life using short form (WHOQOL-BREF).

The objective of this study was to assess Fear-avoidance Belief Questionnaire and Quality of Life in housewives with osteoarthritis of knee joint.

**Aim**

Assessing Fear-avoidance Belief Questionnaire and Quality of Life in housewives with knee osteoarthritis.

**Material and Methodology**

The study will be conducted in DMIMS, Sawangi(M). The study design of this research is going to be cross-sectional study and with observational (survey) study type. Sampling procedure used for this study is purposive sampling method, where every member is selected by chance and every member of the population has an equal probability of being included in the survey.

Sample size - 200

Inclusion criteria - Patient who consent for this study.

Stage 1 -2 of OA according to Kellgren and Lawrence system.

Primary cause of OA.

Exclusion criteria - people who do not consent to participate.

Past history of fractures

Any orthopedic condition making it impossible to perform ADLs.

Secondary causes of OA.

Study duration – 6 months.

Material -

1) Fear- avoidance Belief Questionnaire - The overall test-retest reliability of FABQ was found to be good (ICC=0.97). FABQ Physical activity subscale test-retest reliability (ICC=0.72-0.90). FABQ Work subscale test-retest reliability (ICC=0.8-0.91). Evidence shows that there is correlation between FABQ and Roland & Morris Disability Questionnaire. The correlation co-efficient for the FABQ was 0.52, the FABQ Work subscale was 0.63 and the FABQ Physical Activity subscale was 0.51.

2) Short form WHOQOL- BREF – The WHOQOL-BREF have displayed good discriminant validity, content validity and test-retest reliability. Its sensitivity to change is under assessment. Domain score produced by the WHOQOL-BREF have been shown to correlate at around 0.9 with the WHOQOL-100 domain score.

3) Radiographic image of knee.

**Procedure**

**Permission from head of institute will be taken**

**Ethical clearance**

**Subject will be selected according to inclusion criteria**

**Inform consent shall be filled up**

**The subject will be explained about the study**

**In person administration of questionnaire**

**Data collection**

**Statistical analysis of data to be obtained.**

**figure 1: description of the research protocol.**

Institutional Ethical Committee (IEC) clearance will be obtained prior to the commencement of the study. Participants will be selected as per the inclusion criteria as has been mentioned. Purpose of the study will be explained and inform consent from the participants will be taken. The participants will be given FABQ and short-form of WHOQOL-BREF. After the results have been obtained data will be collected. A statistical analysis will be obtained and the conclusion will be given and a research paper will be created according to the study and published.
Statistical analysis - This is a descriptive study to find frequency and percentage of fear factor and QOL in housewives with knee pain.

Sample size was calculated using the standard formula of

\[ n = \frac{Z_{1-\alpha/2}^2 p(1-p)}{d^2} \]

where,

\[ Z_{1-\alpha/2} \] = standard normal variant error

\[ p \] = expected proportion of population based on previous studies

\[ d \] = absolute error or precision

The data obtained will be assessed using descriptive and inferential statistics. The use of paired-t test will be done for the analysis.

**Expected Result**

The expected outcome will be percentage of fear avoidance belief about physical activity and work which is present in the patients with knee OA. The fear-avoidance scale will be assess depending upon the overall function mobility, ADLs, behaviour, general health, life participation, mental health, motivation, occupational performance, pain, personality, quality of life, self-efficacy, stress and coping. Quality of life will be assessed to find decrease/detoritation in any domain of WHOQOL-BREF due to fear avoidance behaviour. After the completion the study result will be calculated by statistical analysis and will be prepared and published in the form of research paper.

**Discussion**

The research aims to assessing fear-avoidance belief questionnaire and QOL in housewives with knee osteoarthritis.

Our study is supported by a research conducted by Gordon Waddell et al., (1993) in which he concluded that the importance of fear avoidance belief and demonstrated that specific fear avoidance beliefs about work are closely related to work loss because of low back pain\(^{(14)}\). Another study by Marcio Massao Kawano et al., (2015) stated that individuals with OA had low perception of functional capacity, functional limitation and pain in their QOL\(^{(18)}\). In a study by R Piva et al., (2019) interpreted that change in fear avoidance beliefs about physical activity was the strongest predictor of functions and pain outcome\(^{(21)}\). Jack Farr II et al., (2013) deduced that patients with knee OA had negative impact on health related quality of life\(^{(22)}\). An another study by Fatma Fidan et al., (2013) reasoned that patients with knee OA had significant lower QOL as compared to healthy controls\(^{(23)}\).

**Conclusion**

The study will be conducted according to research protocol on the patients with knee osteoarthritis in the institutional setup. In this study we hypothesize that due to fear of increasing pain due to movement there was deterioration in quality of life especially in level of independence. Greater the pain and severity of OA larger will be the deterioration in quality of life. Once the study is completed, data calculation by statistical analysis will be done and published in the form of research paper.

**List of abbreviation -**

1) OA – Osteoarthritis.

2) FABQ – Fear-avoidance Belief Questionnaire.

3) WHOQOL-BREF – The World Health Organization Quality of Life – BREF.

4) AVBRH – Acharya Vinoba Bhave Rural Hospital.

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Screening for Upper Cross Syndrome in Asymptomatic Individuals

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Abstract

Background: Upper cross syndrome has become prevalent in today’s population. This syndrome refers to tightness of muscles such as pectoralis major, upper trapezius and levator scapulae and weakness of deep neck flexors, scalene, rhomboids, serratus anterior, middle and lower trapezius. Poor posture is associated with imbalance created in musculoskeletal system and common condition to be reported as upper cross syndrome. It is commonly seen in people with forward head posture, desk job workers, dentist, beauticians etc. The resulting clinical presentation is complaint of pain in neck and shoulder, cervicogenic headache, hunched upper-back and rounded shoulder. Children are not as proactive today and the rate of the Upper cross syndrome is on the rise. This deviated posture and sedentary lifestyle continues, where it progresses more as the age progresses in adulthood. Aims and Objectives: To screen, detect, early treat as well as prevent upper cross syndrome in young individuals. Materials and Methods: Asymptomatic individual with 20-40 years of age group will be explained about the procedure before commencement of the study. Posture will be screened using Kinect Azure and REEDCO Posture Assessment Scale will be used for evaluation. Assessment of muscle tightness and weakness will be evaluated using muscle length tests and manual muscle testing.

Results: The expected results would include details about prevalence of UCS in asymptomatic individual. The parameters of outcome measures will be analyzed using the statistical test namely students paired T-test.

Conclusion: Based on previous data we assume it is essential to screen asymptomatic individual to looks for signs of Upper Cross Syndrome for early detection, prevention and treatment.

Key Words: Upper Cross Syndrome, Kinect Azure, Muscle Length Testing.

Introduction

There is a strong association of poor posture with imbalance created in musculoskeletal system and is common condition to be reported as upper cross syndrome. Such posture creates muscle tension as well as limits the mobility.¹

In 1988 Dr. Janda put forth an interesting concept by dividing the muscles into two groups: Postural and Phasic. Postural or tonic muscles are important for maintaining upright posture, have tendency to become tight and hypertonic. Phasic muscles, which include almost all other muscles have tendency to become weak and hypotonic UCS is term coined by Janda use for this misalignment. He claimed that maintain a stooped
sitting posture for prolonged period of time is major contributing factor. Other words including proximal or shoulder girdle syndrome are also known. Upper cross syndrome (UCS) is the tightness, over-facilitation of the levator scapulae, pectoralis major and upper trapezius accompanied with weakened, inhibited serratus anterior, deep neck flexors especially scalene, middle trapezius, lower trapezius and rhomboids. Hyperactive neck muscles on one side are counteracts the under active muscles on the opposite side, forming a ‘X’ pattern.

The crossed in upper cross syndrome refers to the crossing pattern of overactive muscle with counter crossing of the under active muscle. When viewed from side an X pattern can be drawn for these two sets of muscles. The overactive muscles forms the diagonal pattern from the posterior neck with the upper trapezius and levator down and across neck to the anterior neck and shoulder with sternocleidomastoid (SCM) and pectoralis major. The other side of X now depicts the underactive muscles, with the deep cervical flexors down towards the mid/lower trapezius, rombhoids and serratus anterior. As we continually assume the seated, forward head posture driven by electronic devices or poor exercise selection and techniques, this X pattern of muscle imbalance will increase.

It is commonly seen in people with forward head posture, desk job workers, dentist, beautician etc. Different movement can cause upper cross syndrome, but most cases develop through poor posture, specifically sitting or standing for prolonged period of time with head forward. Activities promoting this postural position include use of computers and laptops, driving, watching TV, browsing cell phones, texting, use of apps or games, and reading. Alteration of this muscular imbalance occurs through prolonged periods of attaining a posture in the classroom, work place and also attaining a prolonged and sustained posture for other activities like cooking. Children are not as proactive today and the rate of the Upper cross syndrome is on the rise. This deviated posture and sedentary lifestyle continues, where it progresses more as the age progresses in adulthood.

The resulting clinical presentation is a complaint of pain in the neck and shoulder, cervicogenic headache, hunched upper back and rounded shoulder. The common characteristics to diagnose upper cross syndrome include head constantly bent in forward position, increase cervical lordosis (cervical spine is too curved), increased thoracic kyphosis (the outward curvature of spine in the upper back, shoulder and chest is more), The shoulder are elevated, protracted and round and scapular winging (the shoulder blade sits out). Upper cross syndrome can cause many disorders in the body which includes headaches, degeneration of the cervical spine in early stages and the cervical curvature is also lost.

This is often believed that constant maintenance of muscle contraction and fatigue due to weakness of muscle is known to cause chronic type of pain in cervical region. Therefore, neck muscles strength has vital role in stabilizing cervical region. In addition to the shoulder girdle muscle, the Deep Neck Flexors is an essential for controlling and stabilizing the spine and supporting the head’s weight against gravity.

There is need for this Study so as to screen asymptomatic individuals for UCS signs for early detection, prevention and treatment.

**Objective**

To screen, detect and early treatment and prevention of upper cross syndrome in young individuals.

**Methods**

This study will be carried out in Ravi Nair Physiotherapy College, Musculoskeletal OPD, Sawangi (Meghe), Wardha, Maharashtra, India after approval from Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences, Deemed to be University.

**Study Design:** Cross sectional Study.

**Study Setting:** RNPC Musculoskeletal OPD.

**Participant:** All asymptomatic individuals between 20-40 years of age.

**Inclusion Criteria:**

1. Asymptomatic individuals
2. Age group between 20-40 years.
Exclusion Criteria

1. History of Neck pain, Cervical Trauma Or Surgery
2. Known Thoracic Scoliosis
3. Known Rotator Cuff Tear
5. Cervical Radiculopathy

Variables

Outcome Measures

1. **Kinect Azure**: Karen Otte et al studied Accuracy and reliability of Kinect version 2 for clinical measurement of motor function the results showed most of clinical parameters showed good to excellent agreement with absolutes(30 parameters showed ICC(3,1) > 0.7 and consistency(38 parameters showed r > 0.7) and concluded that the Kinect version 2 will serve as a reliable and valid clinical measurement tool.7

2. **REEDCO Posture Assessment Scale**: Gunther, J.et al studied Reliability of two postural tests in postmenopausal women with osteoporosis test-retest and inter-rater. Results showed that in total RPS scores (Kruskal-WallisNonparametric ANOVA, H=6.96, p=.07) no significant Inter-rater difference was found. However, one item (head tiltPosition) was significantly different between therapists (H=8.049, P=.045). There was no significant test-retest difference in total RPSScores. (Wilcoxon t, p=.5217). One of the 10 RPS items (lower back)was significantly different at post-test (Wilcoxon t, p=.0379).and concluded that inter rater differences in Total RPS scores approached significance, and several individual Scale items showed significant inter rater or test-retest differences. Further improvement of the reliability of the RPS should precede Clinical use as an outcome measure.8

Aim is to screen asymptomatic individual for upper cross syndrome by

1. Evaluating Posture
2. Assessing Tight Muscles of head and shoulder.
3. Assessing Weak Muscle of head and shoulder.

1) Postural Assessment:

The posture will be screened in all views Using Kinect Azure and REEDCO Posture Assessment Scale will be used for evaluation.

REEDCO Posture Score (RPS) is a standard posture assessment and is graded in coronal and sagittal view in head-to-foot and it is administered by visual inspection of 10 postural traits viewed laterally (sagittal view including neck, upper back, trunk, abdomen and lower back) or from behind (coronal view including head, shoulder, spine, hips and ankles.

The scores are marked as follows: a value of 0 equals to poor posture or severe deviation, 5 equals fair posture or minimal to moderate deviation, and a value of 10 equals good posture and a score of 59% or less is recorded as postural dysfunction.

Procedure

Before evaluating posture, the participants will be asked to complete the personal data form including the consent form. Next, in order to assess the posture using REEDCO Posture Scale, the female participants will be asked to wear bodysuit, while male participants will be asked to take off their shirt. Markers will be made on anatomical landmarks on 7th cervical spine, bilateral acromion process, 5th lumbar spine and bilateral iliac crest. After that participant will be made to stand on the
postural grid at provided spot with shoes off in three
different direction: front (coronal view), back (coronal
view), and sideways (sagittal view). Then posture will be
analyzed using Kinect Azure scoring will be done using
REEDCO Posture Assessment Scale.

2) Assessment of Tight Muscles.

1. Pectoralis major

Test: Patient lies in supine lying and clasp the hand
together behind the head. The arm is then lowered down
until the elbow touches the examination table. Test is
positive if elbow does not touch the table and indicate
tight pectoralis major muscle.

2. Pectoralis minor

Pectoralis minor functions along with Rhomboids
and levator scapulae to stabilize scapulae during arm
extension Test: Patient is in supine lying. The examiner
places the heel of hand over the coracoid process and
pushes it toward the examination table. Normally
posterior movement occurs with no discomfort to
patient. Test is positive if there is [muscle tissue stretch]
tightness over the muscle during posterior movement.

3. Latissimus dorsai

Test: Patient is placed in supine lying position
and asked to fully elevate the arm through the forward
flexion. If the muscle has normal length the arm will
extend to rest against the examining table. The test is
positive if scapulae does not lie flat against the table
(scapulae remain protracted).

4. Biceps brachi (Arm flexor)

Test: Patient lies in supine lying with shoulder in
extension over the edge of table with elbow flexed and
forearm supinated. The examiner extend the elbow
which would normally have bone to bone endfeel, if
biceps is normal.

The test is positive if full elbow flexion would not
occur and end feel will be a muscular tissue stretch.

3) Assessment of Weak Muscles

1. Deep Neck Flexors (Longus capitis and longus
collis)

Patient position: supine lying with elbow bent and
hand overhead resting on table

Fixation: anterior abdominals must be strong enough
to stabilize to give anterior fixation of thorax to pelvis
before head can be raised by neck flexors.

Test: Flexion of cervical spine by lifting the head
from table with chin depressed and approximated
towards the sternum.

Pressure against the forehead in posterior direction

Weakness: hyperextension of cervical spine
resulting in forward head posture.

2. Triceps brachi (Arm Extensors)

Patient position supine lying

Fixation shoulder in 90° flexion with arm supported
position perpendicular to table.

Test: extension of elbow

Pressure against the forearm in direction of flexion

Weakness: inability to extend the forearm against
gravity.

3. Serratus Anterior

The patient is in standing position with arm flexed
forward in 90 flexion

The examiner applies backward force to the arm

Test is positive: The medial border of scapula will
wing. The patient also has difficulty abducting or forward
flexing arm above 90° with weak serratus anterior.

4. Rhomboids

Patient in prone lying position. Test arm behind the
body so hand is at opposite side back pocket. (Opposite
back pocket). The examiner places the index finger along
and under the medial border of scapula while asking
the patient to push shoulder forward slightly against
resistance to relax trapezius.

The patient is then asked to raise the forearm and
hand away from the body. If rhomboids are normal the
thumb is pushed away from the under scapula.
5. **Middle Trapezius**

Patient position in prone lying with arm abducted to 90 and laterally rotated. The examiner resisting the horizontal extension of arm watching for scapular retraction to occur normally.

Test is positive if scapular protraction occurs and this indicates that the middle fibers of trapezius are weak.

6. **Lower Trapezius**

Patient in prone lying with arm abducted to 120 and shoulder laterally rotated.

The examiner applies resistance to diagonal extension and watches for scapular retraction which normally occurs. Test is positive if scapular protraction occurs, lower trapezius is weak.

**Discussion**

To our knowledge this will be first study to screen asymptomatic individuals for UCS signs for early detection, prevention and treatment.

Practicing poor posture in daily routine for prolong period of time can lead to muscle imbalance resulting in UCS symptoms such as chronic cervical pain, headache and in long term can also result in degenerative changes in cervical spine. Thus is very important to look for signs of UCS.

Moreover this study will employ well established and widely used methods with appropriate reliability and validity to assess muscle tightness and weakness as well as to analyze posture. Although we assume that after complete assessment of individuals they will have better understanding about importance of practicing proper posture in order to prevent early signs of UCS.

**Result**

Upper cross syndrome has been evaluated in symptomatic individuals and has never been evaluated in asymptomatic individuals. The results would include details about prevalence of UCS in asymptomatic individuals. After completion of study result will be calculated by statistical analysis using paired T-test and will be presented in the form of research paper.

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5. Hardeep Oberoi Effect Of Cervicothoracic Taping Along With Stretching Strengthening Exercise Program For Upper Cross Syndrome(2015);1-68
A Novel Research Protocol to Evaluate Psychological Perception Using Brain Gym Exercises in Physiotherapy Students

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Abstract

Brain Gym is an academic kinesiological program that is promoted and applied with a consistent learning purpose that aims at enhancing performance such as memory, psychological perception and cognitive skills. The technique requires the participant to communicate with a series of activities that help the body to understand the primary behaviour and learn how to coordinate the brain and entire body. Brain Gym activities includes of 26 basic motions, which are believed to improve perception and stimulates brain hemisphere by neural re-modeling to facilitate whole brain learning. By ways of balancing both the side of brain, behavioral difficulties, social and intellectual burdens are expected to be reduced.

Aims and Objective: This study aims to evaluate the psychological perception and decrease depression in the undergraduate physiotherapy students.

Method: Here’s a idea we suggest to check the psychological perception with brain gym intervention and the duration for practices comprises of three days a week session, duration of 25 minutes, which is completed in one hour. The depression anxiety stress scale (DASS 21) is used to evaluate disorder which is a valid and reliable tool. This study will be conducted in Ravi Nair Physiotherapy college, Sawangi, Meghe, Wardha. The duration of the study will be six months. The study design is of before after-type with simple randomized sampling.

Result: The data will be analysed using Student paired t test.

Conclusion: The expected outcome includes the detection of stress, depression and anxiety levels which will be evaluated by using DASS-21. Data analysis will be done using students paired t test and conclusion of the study will be published after the results are analysed.

Keywords: Psychological perception, Depression, Stress, Anxiety, DASS-21, Brain gym

Background

It is an interventional type with before-after study design which focuses on the evaluation of the psychological perception after intervention of brain gym exercise. Brain Gym was developed in 1970 as an educational and psychological training system developed and enforced with a specific learning intent. Brain Gym activities includes of 26 basic motions, which are believed to improve perception and stimulates brain hemisphere by neural re-modelling to facilitate whole brain learning (¹). The neural mechanism and white matter connectivity of the brain is influence by the intervention of the exercises (²).

Brain Gym is a instructional curriculum intended to improve social, mental, emotional and physical efficiency and use 26 moves (³) (⁴). According to Brain
Gym literature, the abstract framework on which brain activity is conceptualised is generally simplified and defined along dimensions: laterality, attention and centring (5). Laterality, the synchronization between the brain’s right and left hemispheres, which is considered important for reading, writing, hearing, communicating and being able to walk and think. Focusing, the ability to process information in the brain, which is connected to perception and lack in attention / hyperactivity. The final section, centring, the top and bottom brain parts organized as necessary to combine rational thought with emotion (5). Brain gym intervention aims at the optimization of activity, social participation, and quality of life, as well as the health condition of people with acute and chronic disabilities. The most beneficial way to stimulate the brain is by incorporating kinesthetic and tactile learning, techniques and audio and visual activities to combine the high- and low-brain functions. Brain exercise contributes to sensory integrity, motor learning and a link between brain and body. Recently, brain-inspired methods have gained more popularity in overcoming command and decision-making challenges (6). A motion in the mental workout has been shown to have increased blood circulation and stability, good oxygen levels and healthy metabolism (7).

As per the founders, the daily practice of brain gymnastics leads to activation and development of various sections of the brain, particularly the cortex which allows for smoother and more organized communication between the two sides of the brain for high-level thinking (8). The brain is a complex organ which focuses on motion and according to Hannaford, “activity is necessary for learning” (9). Brain stimulation is very important in neuro-rehabilitation, reducing atrophy, lessen the risk of brain structure lesions and increasing cognitive performance. It is important therapy for elderly patients with depression, as its neuronal advantages have increased for age-related atrophy exacerbated by neuropathology (10). Neuroimaging research has indicated motor development, improve perception and integral approach involve stimulation of domains of auditory perception and more operation of cerebral cortex. The study describes the importance of brain gym exercise in physiotherapy. Exercise can stimulate the brain in such a way that neurons are often in a condition to handle the different data from outside and are capable of responding to a “corporate member” of their duty in compliance with parts of brain activity using the principle of “brain-body link”. Brain Gym is a great source of personal development, enabling individuals to obtain rapid transformations and also improve the quality of life in a different age group.

Many recent experiments have been carried out to determine the efforts required to enhance and stimulate the psychological perception of brain (11). Perception is defined as a essentially relational process in which visual stimuli is translated progressively into projections which serve as the basis for action (12). If a person is nervous or distressed then automatically the energy is pumped into the brain and the brain loses control, thus caused the primed, unexpectedly impaired reaction. Brain gym training can minimize mental stress and encourage brain concentration and perception (13). Students nowadays are under stress from waking up to not having proper sleep in addition to all the pressure of appraisals and examinations (14). This undoubtedly influences the attitude of the student towards learning and academic success (4).

Dr. Chaitanya Kulkarni, Dr. Sanjivani Ramesh Khandale have done a project to detect the effects of brain gym exercise on the attention span of the young students and concluded that the mechanism of reading, recodeng and comprehension has been improved. Also, the effect of these exercises demonstrated an improvement in eye power and hand control, as well as helping to focus on the same focal point while reading and writing concurrently. E Effendy, N Prasant Conducted a analysis demonstrating the effect of brain gymnastics on the population of Nursing Home Care Medan’s and found that brain gymnastics increased PSQI and HARS score levels in the intervention category and reduce stress and depression, in year 2019 here they have found a significant change in life style and improved quality of life in the elderly patient. Keith J. Hyatt in year 2007, conducted a study on school students and finished with a segment explaining Brain Gym exercises to encourage literacy ability, oral reading comprehension, communication skills, pronunciation and learning, self-esteem, memory, analytical thought, imaginative thinking. Brain exercise has seen to be effective in attention improvement, in research and more on it is shown to be very helpful in enhancing concentration, attention, vision and memory as well as
helping to relieve stress.

This research explores the impact on the psychometric characteristics of undergraduate students in the exercise program called as brain gym\(^{(16)}\). Specially because it seems to be a accurate and easy-to-administer scale, the Depression Scale, Anxiety, and Stress-21 are chosen. The DASS-21 consist of three self-report measures used to assess depression, anxiety, and emotional tension\(^{(17)}\). Depression spectrum tests dysphoria. Anxiety assessment tests autonomic activation, psychological anxiety\(^{(18)}\). Stress is the very common condition faced by students nowadays and causes disturbance in their daily lives.

Thanch Duc Tran, Jane Fisher performed a study on the Efficacy of DASS-21 in a cohort of Northern Vietnamese people in rural communities as a screening tool and the study concluded that it can useful in clinical practice and the components such as depression, and stress levels are determined by summing the ratings for the elements in question\(^{(18)}\).

The research aims to calculate the impact of brain gym exercise on stress, anxiety and depression in the student of undergraduate physiotherapy using the scale of DASS-21 as an evaluation method. The brain gym exercise intervention will be performed in all the subjects and then evaluation of the psychological perception will be conducted.

**Methodology**

The study will be conducted in Ravi Nair Physiotherapy College, after the approval the Institutional Ethics Committee (IEC) of Datta Meghe Institute of Medical Sciences, Deemed to be University, Sawangi (Meghe). The study design is of before after-type with purposive sampling.

- **Study design:** Interventional study
- **Sample Size:** 220
- **Duration of study:** 6 months
- **Inclusion Criteria:**
  - Age group - 18 to 23 years
  - Physiotherapy undergraduate students

- Moderate range according to DASS-21 scale\(^{(19)}\)

**Exclusion Criteria:**

- Not-willing to participate
- Student with migraine headache
- Diagnosed with psychological condition
- History of neurosurgery or cognitive damage

**Outcome Measure:**

- Depression, anxiety and stress scale (DASS-21)

- The DASS-21 is a valid and reliable instrument for determining the mental status, with reliability for depression = 0.81, anxiety = 0.89, stress = 0.78 respectively.

  - The DASS-21 has sensitivity = 89% and specificity = 76%.

**Need of Study**

1. To calculate the psychological perception among undergraduate students in physiotherapy.
2. To reduces depression, anxiety and emotional stress in undergraduate students.
3. To assess the effects on psychometric properties in undergraduate students.

**Procedure**

The Institutional Ethics Committee (IEC) Clearance will be obtained priorly. Students will be selected as per the inclusion criteria that has been mentioned. The participants will be informed the aim of the research and will get informed consent. They will be given pre and post interventional assessment using DASS-21 scale, reading will be recorded and the exercise intervention will be given for a month. After the results have been obtained, data collection will be done and statistical analysis will be obtained and the conclusion will be given and a research paper will be created according to the study and published.
BRAIN GYM EXERCISESTREATMENT PROTOCOL:

The Marching it is done as the beginning as warm up, in which subjects stand straight and lift both the legs continuously slight above (20) (21) for a time duration of 1 minute. A Cross Crawl is performed to enhance the coordination between both the sides of the brain, it is done for time duration of 2 minutes (5 sets of 8 repetition). Subject is instructed to stand straight and lift up the leg up to the chest and touch the knee with opposite elbow(22).

Positive Points helps to improves memory and reduce stress levels. The subject is instructed to breathe deeply and gently press the eyeballs with eyes closed (23) for time duration of 1 min (10 repetition).It helps to stimulate the lateral and side to side coordination. A Step Touch is done and it is performed in standing position and the subject is instructed to simultaneously move right legs toward left and left towards right. should be done for a duration of 5 minutes ( 30 repetition).

A Neck Circles exercise helps to reduce stress on the neck muscle, head movement coordination and move the neck in circular motion for a time frame of 2 minutes (3) (20 repetition). A Cook’s Hook-Up helps it stimulates the neurons and enhances the balance between hand and brain(21), where subject is instructed to extent and cross both the hands and fix the fingers together and internally rotate the hands for a time duration: 5 minutes (repetition).

A Brain Button- This exercise is performed and helps to improve the flow of electromagnetic energy and, helps in relaxation. The subject is instructed to palpate belly button with one hand and other hand over the collarbone and perform circular motion with finger for a time duration of 2 minutes(10 repetition)(17). The Thinking Cap helps to enhance learning speed and mood, increase attention span, and improve memory. The subject is instructed to press the top of ear and the bottom continuously(3)duration:1 minute (15 repetition) (24).

A Lazy Eight helps in boosting eye muscle control, balance, and concentration. The subject is instructed to extend the hand and make the figure of Eight horizontally in front(3) Duration: 1 minute (5 repetition). A Trace X helps to increase attention span and improve focus (25), where the subject to close eyes and imagine a figure of “X” and do eyeball movement the duration is 2 minutes (10 repetition).

Expected Result

Once the study is completed statistical analysis will be done using Student paired t test and presented in the form of research paper.

Discussion

The study protocol aims to evaluate the psychological perception in a undergraduate student by using the DASS-21 scale. We hypothesis that there will be improved ability to concentrate and improved focus. The research will help to prevaricate the effectiveness of brain gym exercise on stressed student and help with strategies involved to decrease stress and anxiety.

The DASS-21 is chosen as the aspects addresses the challenges faced by the current generation i.e. stress, anxiety and depression and it entirely evaluate the mental status of a person. The Adaptive practices for students include everyday life activities, effective speech, cognitive skills, adaptability, and learning skills that are observable and based on national norms.
Conclusion

The expected outcome includes the detection of depression and anxiety levels in Undergraduate physiotherapy students which will be done by using DASS-21. Brain gym exercises as an intervention will be taught to the participants to evaluate the psychological perception and solve their intellectual and behavioural challenge.

Ethical Clearance: Institutional Ethics Committee (IEC) of Datta Meghe Institute of Medical Sciences, Deemed to be University, Sawangi (Meghe).

Conflict of Interest: None

Funding support: None

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Gaming addiction to Massively Multiplayer Online Games (MMOGs) and Quality of Life

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Abstract

Context: Massively Multiplayer Online Games (MMOGs) are three dimensional games played on personal computers, mobile phones & video games. Millions of people of all ages worldwide participate in gaming and it has become most popular leisure activity for them which sometimes appear to develop problems as a result of excessive gaming, most commonly among young adults/students. Aim: The aim is to assess the relationship between gaming addiction to Massively Multiplayer Online Games (MMOGs) and quality of life among young adults. Setting and Design: Data collection for the present study was conducted at Private University of Punjab, India in April, 2019. A quantitative research approach with descriptive research design was adopted to assess the relationship between gaming addiction to Massively Multiplayer Online Games (MMOGs) and quality of life among young adults. Subjects and Methods: By purposive sampling technique, 100 samples (18-21yrs) meeting the inclusion and exclusion criteria were selected. Statistical analysis used: Descriptive and inferential statistics were used. Results: Findings of the study revealed that 61% young adults/students were non addicted and 39% were addicted to gaming and quality of life was good among addicted and non addicted young adults/students & there is no significant relationship between gaming addiction to Massively Multiplayer Online Games (MMOGs) and Quality of life at p<0.05.

Conclusion: MMOGs can improve quality of life and playing these online games for long hours may have long term ill effects on various components of health.

Keywords: Gaming addiction, Massively Multiplayer Online Games (MMOGs), Quality of life.

Introduction

Online gaming has been renowned among public due to the betterment in the internet and computer Technology. These online games has gain popularity among young generation at a very fast rate and changed from single user to multiuser or Massively Multiplayer Online Games (MMOGs) in which number of players are there to play the game at same time.\[1\] We cannot turn a blind eye to the ill effects of online gaming as youngsters are mainly involved than the older ones. Researchers claimed that video games are the integral part in this era of computer revolution\[1\]. Massively Multiplayer Online Games (MMOGs) are virtual three-dimensional fantasy games in which people compete with each other and with the computer-generated characters of the particular game world. People used to play these online games on their personal computers, smart phones and video gaming consoles are also a part of this.\[2\] Researches shows that gaming has positive as well as negative effect on health such as it improves cognitive functions, improves mood

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and ward off anxiety and negative effects includes poor academic performance, adverse effects on health and addiction to gaming in youth. According to WHO (World Health Organization), Quality Of Life (QOL) is defined as an individual’s own awareness in their life related to culture and value system in which they live to fulfil their aims, concerns and standards. It is a broad term for various important realm of life. It is intuitive concept having various areas/dimensions that defines a set standard level for emotional, physical, material and social well being. So this act as an remark about an individual’s or about a society that they can compute the various domains of their life. Undoubtedly such games are a big source of endless entertainment but they also affect the life of people playing these games on regular basis, both in positive and negative aspects.

A great number of scientists and psychologists have claimed that these video games have many advantages and the important and main is that it is making people astute. According to a Psychologist in University of Wisconsin (C. Shawn Green), “Video games change our brain”. Because playing these games help us in recasting of our brain physiological structure in same way as it is recasted during reading, navigating, playing a piano.

In India, most of the gamers are reported to play for more than ten hours straight, and go to sleep without showing any visible signs of their general health being affected, but other players abandon their basic body requirements. They are reported to have lack of personal hygiene, lack of proper nutrition, lack of exercise, and lack of sleep also. In worst case scenarios, people have played these games for so long that they developed deep vein thrombosis (a blood clot) which further resulted as heart attack in them and ultimately death & also affecting their overall health and thus quality of life.

In this modern era we cannot ignore the ill effects of computers on our physical and psychological health. Most of the time the young generation is seen to be more engaged than the older ones with computers. Researchers also said that the development in modern technology is due to computer revolution and computer games are important and integral key aspect of them.

Now days living an ordinary life is not commendatory, but revamping the quality of our living is more recommended and people pay more prominence in improving their life quality wise and consider this as a fundamental goal. This is one of the important and considered as a key indicator since it includes different domains like Physical Health, Psychological, Social relationships and Environment. So basically computing quality of life will help us to identify the societal needs, adjustment programs and budgets. Today, Massively Multiplayer Online Games have become very prevalent among people of different age groups especially adolescents and young adults. Gaming is one the fastest growing and the most economic industry these days. According to a survey conducted by statista.com, it has been found that the total number of online gamers worldwide has massively increased from 1.6 billion in 2014 to 2.2 billion in 2018. They have also estimated an increase of up to 2.6 billion by the year 2021.

Previous studies on gaming addiction to MMOGs and its relationship with quality of life have concluded that addiction to such games has various ill-effects on overall health and is progressively worsening the quality of life of the gamers. Moreover, since India is a country where the rate of playing MMOGs is increasing significantly in young adults and adolescents as it is becoming their favourite leisure activity and many of the youngsters keep themselves busy and diverted due to this. There were very few researches found on this topic and the objective of this study is to find out the level of addiction to MMOGs among young adults and its relationship to the quality of life. No doubt is there that playing this for shorter duration will relax your mind and boost your mood but excess of this can cause serious ill effects on your health. We have studied the published literature regarding this topic and to determine the impact of playing MMOGs upon the quality of life of emerging adults, and young adults particularly at our college level and to critically appraise the evidence was main aim of the study.

### Subjects and Methods

#### Study Setting & Samples

The present research was conducted in private university of Punjab India. Subjects were taken from various departments of the university who used to play
these MMOGS from more than 6 months and falls under the age of 21 years. Only those young adults/ students were considered for participation who were interested to take part in study and all provided written consent before that.

**Assessment Instruments**

In present study, two tools were used, one was Gaming Addiction Scale (GAS) by Lemmens, Valkenberg and Peter to find out the gaming addiction among young adults which consists of total 7 questions and each is measured on a 5 point Likert scale. These seven questions consists of seven criterias of computer addiction such as Salience, Tolerance, Mood modification, Relapse, Withdrawl, Conflict and Problems and if 4 or more criterias are met in a person then the person is diagnosed as addicted (given in DSM section on Gambling). The second tool used was WHO- QOL BREF (World Health Organisation- Quality of Life ) Questionnaire which checked the quality of life among these addicted and non addicted diagnosed subjects and this tool consists of total 26 questions sub divided into four domains such as Physical Health contains seven questions about physical health, Psychological Domain contains six questions, Social relationships Domain consists three questions and Environment domain consists 8 questions respectively and two were general questions not considered under any domain with Maximum Score=108 Minimum Score=36.

**Study Design & Data Collection**

Descriptive research design was used to assess the relationship between gaming addiction to Massively Multiplayer Online Games (MMOGs) and Quality Of Life (QOL) among young adults/ students and quantitatively, data was collected and converted into numerical form to make statistical calculations & reveal results. Non-Probability Purposive sampling technique was used to draw 100 samples from the population after fulfilling the inclusion and exclusion criteria, subjects were provided with Gaming Addiction Scale (GAS) to find out the gaming addiction and WHO QOL BREF (World Health Organisation Quality of Life ) Questionnaire was used to check the quality of life among them. Nature and purpose of the study was explained to the young adults/ students and informed consent was taken from them.

**Results**

**Table no. 1: Frequency and Percentage distribution of gaming addiction to Massively Multiplayer Online Games (MMOGs)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Gaming Addiction Criteria (DSM)</th>
<th>Gaming Addiction Scoring</th>
<th>f (%)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Addicted</td>
<td>4 or &gt;4</td>
<td>39 (39%)</td>
<td>4.95</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>Non-addicted</td>
<td>&lt;4</td>
<td>61 (61%)</td>
<td>1.70</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Maximum=7 Minimum=0

Table 1 depicted that majority of the population (61%) was not addicted to Massively Multiplayer Online Games (MMOGs).
Table no. 2: Frequency and percentage distribution of Quality of life among Addicted and Non Addicted young adults/ students.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Category Score</th>
<th>Addicted (n = 39)</th>
<th>Non Addicted (n= 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>f (%)</td>
<td>f (%)</td>
</tr>
<tr>
<td>1</td>
<td>EXCELLENT (93-108)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>VERY GOOD (79-92)</td>
<td>02 (5%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10(26%)</td>
<td>16 (26%)</td>
</tr>
<tr>
<td>3</td>
<td>GOOD (65-78)</td>
<td>26 (67%)</td>
<td>42(69%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>01 (2%)</td>
<td>02(3%)</td>
</tr>
<tr>
<td>4</td>
<td>AVERAGE (51-64)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>POOR (50-36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum Score=108</td>
<td></td>
<td>Minimum Score=36</td>
</tr>
</tbody>
</table>

Table 2 showed that majority of the Non Addicted young adults/ students (69%) had good level of quality of life.

Table no. 3: Domain wise Mean and SD of Quality of life among addicted and non-addicted young adults/ students.

<table>
<thead>
<tr>
<th>DOMAINS</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Addicted (n=39)</td>
<td>Non-addicted (n=61)</td>
</tr>
<tr>
<td>Domain 1 (Physical)</td>
<td>22.56</td>
<td>22.18</td>
</tr>
<tr>
<td>Domain 2 (Psychological)</td>
<td>19.15</td>
<td>19</td>
</tr>
<tr>
<td>Domain 3 (Social Relationships)</td>
<td>9.83</td>
<td>9.98</td>
</tr>
<tr>
<td>Domain 4 (Environment)</td>
<td>25.17</td>
<td>23.91</td>
</tr>
</tbody>
</table>

Table 3 depicted that among the addicted young adults/ students, the most affected domain was Environment domain with the highest mean score (25.17) and SD (4.50). Among non-addicted young adults/ students the most affected domain was Environment domain with the highest mean score (23.91) and SD (4.46).
Table no.4: Relationship between Gaming addiction to Massively Multiplayer Online Games (MMOGs) and Quality of life among Addicted and Non-addicted young adults/ students.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Domain</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Addicted (n=39)</td>
<td>Non-addicted (n=61)</td>
</tr>
<tr>
<td>1</td>
<td>Domain 1 (Physical)</td>
<td>0.060</td>
<td>-0.055</td>
</tr>
<tr>
<td>2</td>
<td>Domain 2 (Psychological)</td>
<td>-0.249</td>
<td>-0.188</td>
</tr>
<tr>
<td>3</td>
<td>Domain 3 (Social Relationship)</td>
<td>0.008</td>
<td>0.236</td>
</tr>
<tr>
<td>4</td>
<td>Domain 4 (Environment)</td>
<td>0.023</td>
<td>0.104</td>
</tr>
</tbody>
</table>

p<0.05

Among 39% of addicted young adults/ students in terms of quality of their life, 67% had good level, 26% had very good level, 5% had excellent level, and 2% had average level and none of the young adults/ students had poor level of it. On the other side there were 61% of non-addicted young adults/ students and in terms of their quality of life, 69% had good level, 26% had very good level, none of the young adults/ students had excellent level, 3% had average level and 2% of the young adults/ students had a poor level of it.

A similar study was there on the influence of internet based activities on quality of life which concluded that 14.1% participants believed that their quality of life could be improved by limiting access to internet usage for different activities including playing online games which also emphasized on the fact that playing internet based gaming also affected their quality of life. In our study it has been found that there is weak positive correlation between gaming addiction and quality of life. Contrary to Mr. Pontes’ study, environment domain was found to have been affected the most among subjects in our study.9
In order to assess the relationship between gaming addiction to Massively Multiplayer Online Games (MMOGs) and quality of life among addicted and non-addicted young adults/students, Karl Pearson’s correlation coefficient was used in order to find a relationship between the gaming addiction scores of addicted and non-addicted young adults/students with different domain wise score of WHOQOL-BREF.

Among 39 addicted young adults/students, there was weak positive correlation between gaming addiction score and domain 1, 3 and 4 whereas a weak negative correlation between gaming addiction score and domain 2 at p<0.05. Among 61 non-addicted young adults/students, there was a weak negative correlation between gaming addiction score and domain 1, 2 whereas a weak positive correlation between gaming addiction score and domain 3, 4 at p<0.05. A similar study was conducted on the relationship between computer games and quality of life and it was concluded that playing computer games for long hours can have negative impact on health which may lead to poor quality of life. Similarly, our study concluded that gaming addiction may hamper many domains of quality of life primarily the Environment domain.

Conclusion

In the present study, among a sample of 100 young adults/students, 39 were found to be addicted to Massively Multiplayer Online Games (MMOGs) and 61 were found to be non-addicted. 68% of the population had a Good level of quality of life. There was a weak positive correlation between gaming addiction score and different domains of quality of life (Physical, Psychological, Social relationships and Environment) among young adults/students.

Acknowledgement: We are thankful to our University authorities, our Principal and all the faculty members who helped us at various steps of our research process and most important we are thankful to all the enrolled participants for their valuable time and feedbacks.

Financial Support and Sponsorship: Nil.

Conflict of Interest: There is no conflict of interest.

Ethical Clearance: Ethical clearance was taken from Research Ethical Committee of University.

References


8. Dolatabadi, N. K., Eslami, A. A., Mostafavi, F.,


Knowledge, Attitude, and Practice about Informed Consent amongst Resident Doctors at Rural Medical Institute of Central India

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Abstract

Informed consent is the process by which the treating health-care provider discloses appropriate information to the patient so that the patient may make a voluntary choice to accept or refuse treatment. There are few studies done amongst the medical residents in India about informed consent. The residents are the stepping stone of the Medical profession, it is proposed that the perception about the informed consent amongst the residents shall be sought. Hence, the study was undertaken with the aim, to appraise the knowledge, attitude, and practices of residents of all three years toward ‘informed consent taking’ with the objectives of assessing and comparing the knowledge, attitude and practices of obtaining informed consent. The survey questionnaire was circulated and data was collated. It was developed in Knowledge, Attitude and practice domain and analysis was done. Based on the result, it was concluded that, in all three domains, there was ascendancy of Knowledge, Attitude and Practice in three years of resident doctors.

Key words: Informed consent, Knowledge, Attitude, Practice, Resident Medical Doctors.

Introduction

Consent is a lawful right of a patient that decides their involvement in clinical procedures. The meaning of consent is that patients not to be touched or in nevertheless treated without their permission, it considers like an endorsed inquiry for their protection. Consent is a thoughtful agreement between doctor and patient, and in the health-care sector, it gives moral values to maintain the dignity and sanctity of the profession. The concept of consent is an endeavour by which the patient can take part in clinical judgment concerning their treatment and protect every patient against any litigation. Knowledge and approach of consent are foremost important due to encroachment in clinical procedures in the medical field.

Informed consent is the process by which the treating health-care provider discloses appropriate information to the patient so that the patient may make a voluntary choice to accept or refuse treatment. It originates from the legal and ethical right the patient has to direct what happens to his/her body and from the ethical duty of the physician to involve the patient in his/her health care.

Some of the revealing facts about Informed consent for the subjects of Clinical trial can be depicted as the basic ethical principle behind informed consent legalities is to protect the autonomy of human subjects which states that welfare and interests of a subject participating in clinical research are always above the society’s interests and welfare.

ICMR guidelines stipulate that volunteers must be provided all information on physical and psychological risks as well as moral implications of the research. They also stated that research should include an inbuilt mechanism for compensation to cover “all foreseeable and unforeseeable risks” a fact rarely mentioned. 
It is an obligatory duty of health-care professionals to help their patients for taking conversant decisions regarding treatment procedures. Thus, knowledge and approach of consent are foremost important due to encroachment in clinical procedures in the medical field as well as it is also important for the general population.

Nowadays there are physical attacks on practitioners about bad patient outcomes. According to a study by the Indian Medical Association, over 75% of doctors have faced violence at work. There may be multiple reasons behind this but the study by Neeraj Nagpal stated that one of the reasons is consent taking (other reasons are poor communication or Meagre health budget and poor-quality healthcare etc). It also suggested that proper steps should be taken to avoid such incidences like taking proper informed consent (eg. some technical issues like consent in their language, explaining them in detail in their language, for children taking consent from their parent, etc).

There are few studies done amongst the medical residents in India about informed consent. The residents are the stepping stone of the Medical profession, it is proposed that the awareness about the informed consent amongst the residents shall be sought.

Hence, the study was undertaken with the aim, to appraise the knowledge, attitude, and practices of residents of all three years toward ‘informed consent taking’ with the objectives of assessing and comparing the knowledge, attitude and practices of obtaining informed consent amongst all three-year resident doctors of Medical postgraduate students of Medical Institute of central India.

**Methodology**

The study was descriptive cross-sectional survey for resident doctors. Sample size was calculated by complete enumeration method. All the postgraduate students of broad specialties of Medicine faculty in the postgraduate departments were included. The departments included were Medicine, Surgery, Obstetrics and Gynaecology, Paediatrics, Orthopaedics, and Anaesthesia.

Survey questionnaire was prepared as study tool which was pre-validated by the experts of medical education. It was based on 3-point Likert scale.

Survey questionnaire was prepared under three headings as follows:

1. Describing the knowledge regarding informed consent taking including 9 questions
2. Describing the Attitude regarding informed consent taking including 5 questions
3. Describing the Practice regarding informed consent taking including 5 questions

The study was conducted after obtaining the Institutional Ethics clearance.

The data was analysed using SPSS version 20. A Chi-square test was used to compare Junior Residence of 1st year 2nd year and 3rd year doctors to find out the statistical significance.

**Observation and Results**

There were total 216 resident doctors from all 3 years working in the six broad specialty departments of Medical faculty. The demographic profile depicted 115 (54%) males and 101 (46%) females. Out of 216, the first year, second year and final year resident doctors were 70, 72, and 74 respectively.

<table>
<thead>
<tr>
<th>SN</th>
<th>Broad specialization</th>
<th>Number of resident doctors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Medicine</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>Surgery</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Obstetrics &amp; Gynaecology</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>Paediatrics</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Anaesthesia</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>Orthopaedics</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>216</td>
</tr>
</tbody>
</table>

The overall response rate was 64% (139) and the distribution were 64% (45/70) for 1st year, 68% (49/72) for 2nd year and 64% (45/74) for final year resident doctors.
The survey was sent online as google forms and data collected and collated. The depiction of the same is described in the following tables.

**Table 2. Knowledge about informed consent amongst resident doctors (N=139)**

<table>
<thead>
<tr>
<th>SN</th>
<th>Questions</th>
<th>Options</th>
<th>JR-I n=45</th>
<th>JR-II n=49</th>
<th>JR-III n=45</th>
<th>Total N = 139</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1.</td>
<td>Do you know what is an informed consent?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2.</td>
<td>Do you know what is a verbal consent?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q3.</td>
<td>Do you know what is a written consent?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4.</td>
<td>Do you know, the patient’s signature be taken after verbal consent?</td>
<td>Yes</td>
<td>34</td>
<td>36</td>
<td>45</td>
<td>115</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>11</td>
<td>13</td>
<td>0</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q5.</td>
<td>Should the patient’s consent be taken before treatment?</td>
<td>Yes</td>
<td>36</td>
<td>40</td>
<td>42</td>
<td>118</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>09</td>
<td>09</td>
<td>03</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q6.</td>
<td>Do you know that consent should be obtained for disabled/child patient?</td>
<td>Yes</td>
<td>38</td>
<td>49</td>
<td>45</td>
<td>130</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>09</td>
<td>0</td>
<td>0</td>
<td>09</td>
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<td>45</td>
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<td>45</td>
<td>139</td>
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<td></td>
</tr>
<tr>
<td>Q7.</td>
<td>Does patient’s consent help with the treatment?</td>
<td>Yes</td>
<td>31</td>
<td>38</td>
<td>39</td>
<td>108</td>
<td>P &lt; 0.05</td>
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<td>45</td>
<td>139</td>
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</tr>
<tr>
<td>Q8.</td>
<td>Are you aware that one copy of the informed consent form should be given to the patient if asked for?</td>
<td>Yes</td>
<td>40</td>
<td>45</td>
<td>42</td>
<td>127</td>
<td>P &gt; 0.05</td>
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</tr>
<tr>
<td>Q9.</td>
<td>Are you aware of the Consumer Protection Act?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
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<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
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</tr>
</tbody>
</table>
Table 3. Attitude of resident doctors about informed consent (N=139)

<table>
<thead>
<tr>
<th>SN</th>
<th>Questions</th>
<th>Options</th>
<th>JR-I</th>
<th>JR-II</th>
<th>JR-III</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1.</td>
<td>Have you been taking the consent from the patient before any treatment?</td>
<td>Yes</td>
<td>40</td>
<td>49</td>
<td>45</td>
<td>134</td>
<td>P &gt; 0.05</td>
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<td>0</td>
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</tr>
<tr>
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<td>Not Sure</td>
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<td>0</td>
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<td></td>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q2.</td>
<td>Do you take signatures of the patient, even if it is a verbal consent?</td>
<td>Yes</td>
<td>40</td>
<td>46</td>
<td>42</td>
<td>134</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>05</td>
<td>03</td>
<td>03</td>
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<td>0</td>
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<td>Total</td>
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<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q3.</td>
<td>Do you take the consent of the patient/relative, for surgical procedure?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
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<tr>
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<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q4.</td>
<td>Do you take the consent of the patient/relative, for noninvasive procedure?</td>
<td>Yes</td>
<td>31</td>
<td>38</td>
<td>45</td>
<td>114</td>
<td>P &lt; 0.05</td>
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<tr>
<td></td>
<td></td>
<td>Not Sure</td>
<td>14</td>
<td>11</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q5.</td>
<td>Do you provide a copy, if patient asks for a copy of the consent form?</td>
<td>Yes</td>
<td>40</td>
<td>49</td>
<td>45</td>
<td>134</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>0</td>
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<td>11</td>
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<td>Total</td>
<td>45</td>
<td>49</td>
<td>45</td>
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</table>

Table 4. Questions regarding practices of taking informed consent amongst resident doctors (N=139)

<table>
<thead>
<tr>
<th>SN</th>
<th>Questions</th>
<th>Options</th>
<th>JR-I</th>
<th>JR-II</th>
<th>JR-III</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1.</td>
<td>I inform patients about their medical condition and procedures of the treatment?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q2.</td>
<td>I always answer the patients queries regarding treatment?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
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<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q3.</td>
<td>Do you inform your patient about possible consequences if he/she refuses the treatment?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
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<td></td>
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<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
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</tr>
<tr>
<td>Q4.</td>
<td>Do patients receive a copy of signed consent form?</td>
<td>Yes</td>
<td>35</td>
<td>45</td>
<td>45</td>
<td>125</td>
<td>P &gt; 0.05</td>
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<td>49</td>
<td>45</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Q5.</td>
<td>Do you inform patients about the length of their hospital stay?</td>
<td>Yes</td>
<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
<td>P &gt; 0.05</td>
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<td>45</td>
<td>49</td>
<td>45</td>
<td>139</td>
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</table>
Table 2, 3, and 4 depicted that the knowledge, attitude and practices of Informed consent amongst all 3 years of residency of postgraduate trainees was adequate in most of the aspects barring few areas and it was increasing as they go to higher levels of their training period.

**Discussion**

Informed consent deemed as an integral part of the doctor and patient rapport as well as delivery of successful treatment. Knowledge and attitude should always in equanimity; once knowledge gets better, attitude and practices will automatically improve.

From the data of the study, it was found that the overall knowledge and attitude of resident doctors for informed consent was adequate. The ascendancy in all three domains was seen in the three years of their training. It was found that the knowledge, attitude and practice with regard to informed consent in medical practice was more in 2nd year in comparison with 1st-year resident doctors and it was more in 3rd-year resident doctors when compared to resident doctors. It was observed that there was an improvement in knowledge, attitude, and practice in 3rd-year resident doctors, but not significant except in few areas like “does patient consent help with treatment” or “do you take patient consent for non-invasive procedures” where it was significant.

In knowledge domain the observations could be inferred as; all three years of the residents were having significant knowledge of informed consent like various types of consent, the importance of signature, consent in disabled patients, and the Consumer Protection Act CPA act. Except for one area that the consent helps in the treatment where the p-value was significant. The first-year students were not sure about this.

The study by Gayatri Gupta et al studied the knowledge of informed consent in consultants and residents and found sufficient knowledge of informed consent. The results of our study were in accordance with their results. Anshika Khare et al stated similar results. Their study proved that knowledge regarding informed consent was superior in both medical and dental practitioners. Our study was also having similar results. The study by Vivek Gupta et al studied the knowledge and attitude of informed consent amongst dental practitioners and stated that dental practitioners had an unbalanced knowledge about informed consent. Shamsa Zafar et studied the awareness of informed consent amongst final year students in Pakistan and found it deficient.

In attitude domain the observations could be inferred as: all students had the attitudinal aspects with regard to informed consent except for taking the consent of the patient/relative for the non-invasive procedure like injections or vaccinations. About 18% of students were not sure about it. Anshika Khare et al revealed in their study that attitude was often not as good as needed. In our study, we get positive results for the attitudinal aspect of informed consent.

The study by Vivek Gupta et al studied the knowledge and attitude of informed consent amongst dental practitioners and stated that dental practitioners with the attitude toward its use in the clinical setting were found very dissatisfactory. The study by Biswajit Chatterji in undergraduate medical students revealed that students generally agreed and subscribed that awareness of ethics was important.

In practice domain the observations could be concluded as; all 3 years of residents are practicing the procedures or processes of informed consent necessary for patient care. The same has been reflected in the study done by Patond et al. Only in one aspect where receiving the copy of informed consent, 10% of JR1, and JR 2 was not sure but the value was nonsignificant. Anshika Khare et al revealed in their study that practicability of the knowledge was often not as good as needed. In our study, the students knew the practical use of informed consent.

The overall findings in the study may be attributed to the institutional policy that, at the commencement of training, the Postgraduate induction program is conducted at University level for all new entrants, where ethicality in medical practices are explained in detail. The same has been reflected by Ashish Jain et al.

Hence, we could conclude that there was an improvement in knowledge, attitude, and practice in 3rd-year resident doctors as compare to 1st and 2nd year. The only limitation for this study was inclusion of only 6 broad specialty. Hence, we recommend to conduct such
type of study in all clinical departments as well as at
different medical colleges of different states of India.

**Ethical Clearance:** Taken from Institutional
Ethics Committee, DMIMS (DU) Sawangi (M) dated
13.12.2019

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Consent: Is It Practiced What Is Being Preached?
J Indian Acad Forensic Med. October-December
2015, Vol. 37, No. 4
Total Elbow Arthroplasty in a Rare Case of Giant Cell Tumour of Humerus: A Case Report

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Abstract

Giant cell tumour forms 4 percent - 5 percent of all bone tumours & 20 percent of all benign bone tumours. It is responsible for 20 percent of bone tumours in China. It is usually single, aggressive locally & accounts for 5 percent of metastasis & 1 percent - 3 percent with malignant changes. Most commonly it is found in people aged 30 to 40 years and in the long bones mainly the meta-epiphyseal region. It is most commonly affects the distal femur, distal radius, proximal tibia and proximal humerus. But the occurrence in the distal humerus is rare. A 50 year old male patient who came with complaints of pain and swelling over right elbow since 3 years. Patient gave history of trauma to right elbow as he met with a road traffic accident. After few days patient noticed swelling which was insidious in onset and gradually kept increasing in size. Patient also had pain in right elbow which was insidious in onset and increased in severity daily. Pain was managed with total elbow arthroplasty and elbow range of motion improved after the surgery. Patient was managed with total elbow arthroplasty and wide resection of tumour which has good functional outcomes, less complications and lower recurrences rates. Patient was followed up at 18 – month and free range of motion of 10º to 110º without pain along with no proof of reoccurrence was noted.

Keywords: Giant cell tumour, Humerus, Arthroplasty, Elbow prosthesis, Rehabilitation.

Introduction

Giant cell tumour (GCT) forms 4 percent to 5 percent of all bone tumours and 20 percent of all benign bone tumours (1). GCT is responsible for 20 percent of bone tumours in China (2). It is usually single, aggressive locally & accounts for 5 percent of metastasis & 1 percent - 3 percent with malignant changes (3). Most commonly it is found in people aged 30 to 40 years and in the long bones mainly the meta-epiphyseal region (4). It is most commonly affects the distal femur, distal radius, proximal tibia and proximal humerus. But the occurrence in distal humerus is rare (5).

Histologically, giant cell tumour is divided into 3 types:

a) Multi-nucleated giant cells resembling osteoclasts

b) Mononuclear histiocytic cells

c) Neoplastic stromal cells – main proliferating cells

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Radiologically, giant cell tumour presents as radiolucent lesion without calcifications which are centrally placed inside the bone giving it soap bubble appearance (6).

Giant cell tumour is usually treated with intralesional curettage either with bone cement packing or bone grafting. If the lesion is close to the articulating surface, subchondral bone grafting is done. With these procedures the reoccurrence rate ranges from 10 percent to 40 percent (7).

**Patient and Observation**

In November 2019, A 50 year old male who came with complaints of pain and swelling over right elbow since 3 years. Patient gave history of trauma to right elbow as he met with a road traffic accident. After few days patient noticed swelling which was insidious in onset and gradually kept increasing in size. Patient also had pain in right elbow which was insidious in onset and increased in severity daily.

On examination a diffuse swelling was noted around the distal humerus. The local temperature was raised, and tenderness was noted over and surrounding area of the swelling. The swelling was extending from 18cm distal to tip of Greater tuberosity right side; up to 17cm proximally from radial styloid process; 55cm in circumference at most prominent part with 25cm vertically and 20cm horizontally (Figure 1,2). Margins of swelling are ill defined with soft, doughy, bony hard mixed consistency present. Over lying skin was non-pinch able. A fixed flexion deformity of 60º, along with range of motion of 30º to 80º was noted which was painful. Supination and pronation was painful and restricted.

Radiographs showed an expansile osteolytic lesion in the olecranon fossa of the humerus with involvement of radial and ulnar head resembling a ‘soap bubble’ like appearance. Magnetic resonance imaging (MRI) depicted a well-defined, expansile mass, with altered signal intensity, involving the articular surface of the distal end humerus causing cortical break with extra-osseous extension into adjacent soft tissue; displacing adjacent muscles and the vessels. Patient was advised cytology and the report was suggestive of Giant cell tumour.

The patient was operated with total abscission of the distal humerus along with total elbow arthroplasty. A cemented ‘sloppy-hinge’ total elbow prosthesis (Bakshi, Sis Ortho, Bangalore, India) made of stainless steel was used. A high arm tourniquet was used during the procedure to avoid excessive blood loss.

A postero medial skin incision taken, soft tissues dissected & the ulnar nerve along with blood vessels was identified and isolated. The excision of tumour was done by sharp dissection with a edge of normal tissues & precaution was taken that the capsule was not damaged. The olecranon process along with radial head was excised and removed. The triceps attachment was not resected during the procedure (Figure 3).

Appropriate sized cemented elbow prosthesis was used and inserted (Figure 4). Hemostasis was achieved after removal of tourniquet. Irrigation was done with 3 litres of normal saline and wound was sutured in layers. A suction drain of size FG 10 was inserted. The limb was kept in full extension with an above-elbow slab. The resected tumour mass was sent for histo-pathological examination and the report confirmed the diagnosis of Giant Cell Tumour of bone. After one week of post-operative time, rehabilitation was initiated which comprised of active and passive movement within pain free range and with gradual progression. Stitches were removed two weeks later and was discharged with home program exercises and advised for monthly follow-ups. At 8th month follow up patient had a free range of motion of 10º to 110º without pain along with no evidence of reoccurrence was noted.

**Timeline:**

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
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<tr>
<td>First encountered discomfort</td>
<td>2016</td>
</tr>
<tr>
<td>Date of injury(accident)</td>
<td>09-11-2019</td>
</tr>
<tr>
<td>Date of surgery</td>
<td>11-11-2019</td>
</tr>
<tr>
<td>Date of physiotherapy rehab</td>
<td>18-11-2019</td>
</tr>
<tr>
<td>Date of suture removal</td>
<td>30-11-2019</td>
</tr>
<tr>
<td>Date of follow-up</td>
<td>03-08-2020</td>
</tr>
</tbody>
</table>
Figure 1: Figures shows giant cell tumour of right elbow (lateral, anterior and posterior view)

Figure 2: Figure shows pre-operative X-ray anterior and lateral view of right elbow showing soap bubble appearance suggestive of giant cell tumour.

Figure 3: Intra-operative images showing a) Excised giant cell Tumour with part of distal humerus, b) Isolated median and ulnar nerves, c) Implant in-situ.
Discussion

Giant cell tumour of bone most commonly occurs as anomalous, expansile lytic lesion, with thinned cortex and in later stages may breach the cortex of bone (8). Giant cell tumour results in expansile remodelling of the overlying bone; cortex overlying the tumour mass remains intact. Sometimes tumour contains foci of dystrophic mineralization (9). The most important histological and morphologic feature is multi-nucleated giant cells which may have 100 nuclei with prominent nucleoli. The distinguishing feature of giant cell tumour from other bone tumours or the osteogenic lesions that have benign osteoclast is that the surrounding mononuclear and multinucleated cells that are small in size have nuclei similar to those found in the giant cells.

Surgery is the management of choice. Cementing and/or bone grafting along with curettage is done (10). If the tumour invades the bone cortex along with the joint, an en bloc (total) resection with reconstruction of total joint is the treatment modality of choice. The complications rates among these are very high and are mainly used as salvage procedures if the total elbow arthroplasty fails (11). Excellent functional outcomes and less recurrences rates have been seen in wide resection of tumour and total elbow arthroplasty (12). A well-structured rehabilitation is a vital part in the successful recovery of the patient outcomes to restore patient’s functions (13). Total elbow arthroplasty is another option as it has good functional outcomes and pain relief with less complication rates.

Conclusion

Giant cell tumour is rare condition in lower end humerus and the prompt and accurate diagnosis of the same and total elbow arthroplasty management led to a successful and acceptable functional improvement for our patient. Physical rehabilitation post-surgery in such replacement procedures stands a important aspect in the positive outcomes.

Declaration of Patient Consent:

The authors certify that appropriate consent form was obtained from the patient. In the consent form, the patient has given his consent for his clinical information to be published in the journal. The patient understands that their personal information will not be described.
Author’s Contribution: 

All authors made the best contribution for the concept, assessment and evaluation, data acquisition and analysis and interpretation of the data.

Financial Support and Sponsorship: No Funding Support.

Conflicts of Interest: No conflicts of interest.

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Relationship between Working Tenure and Working Posture with Musculoskeletal Grievance in Batik Madura Workers

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Abstract

Introduction: Batik is one of the cultural heritages of Indonesian people. Batik is the result of acculturation of Javanese, Hindu and Islam which is written on a sheet of fabric. The process of making batik fabric takes a long time. This condition causes many batik workers experience work related disease, such as musculoskeletal grievance. This research aimed to analyze the relationship between working tenure and working posture with musculoskeletal grievance in Batik Madura workers.

Methods: This research was an observational research with a cross sectional design. The research population was all Batik Madura workers. The sample in this study was 61 Batik Madura workers. The variables researched included working tenure, working posture and musculoskeletal grievance.

Data were collected by observation, filling out research questionnaires, and Nordic Body Map (NBM). The data analysis used was the Spearman correlation.

Results: There was no relationship between working tenure with musculoskeletal grievance with a value of $p = 0.837$ and there was relationship between working posture with musculoskeletal grievance with a value of $p = 0.000$.

Conclusion: The working tenure didn’t have relationship with musculoskeletal grievance. The working posture had a relationship with musculoskeletal grievance.

Keywords: working tenure, working posture, musculoskeletal grievance

Introduction

Batik is one of many cultural heritages in Indonesia has confirmed by UNESCO in 2009 as a masterpiece of oral and intangible human heritage to Indonesia. Therefore, every October 2 is designated as the national batik day on which every community in Indonesia wears a batik shirt. Batik fabrics in Indonesia have various motifs and colors. Batik motifs are created with messages and hopes that are sincere and noble, so that they will bring goodness and welfare as well as happiness to the wearer1. Occupational Safety and Health is a thought and effort made to ensure wholeness and perfection both physically and spiritually so that workers can carry out work activities safely, comfortably, and achieve physical endurance, work power, and a high level of health2. Occupational safety and health must be applied in various types of industries, both formal and informal industries. However, occupational safety and health efforts in the informal industry in Indonesia still lack special attention to informal industry owners. This condition causes workers in informal industries in Indonesia to experience many occupational accidents and work related diseases.

Work related disease is a disease caused by work, work tools, materials, processes, and the work

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DOI Number: 10.37506/ijfmt.v15i1.13379
environment. Work related disease also defined as a health disorder, both physically and spiritually arising from or exacerbated by work activities or conditions work related. Work related disease doesn’t get intervention can cause a decrease in worker productivity which will have an impact on industries in Indonesia experiencing losses. Workers in informal industries such as the batik industry have a great potential to experience work related disease. Data of International Labor Organization showed that every year there was 2.78 person died due to work related disease. Data of Ministry of Health in Indonesia showed that 26.78% of workers in Indonesia experience diseases due to the work they do. One of the work related diseases that can be experienced by batik workers is Musculoskeletal Disorders (MSDs).

Musculoskeletal Disorders (MSDs) is disturbance experienced by workers on muscles, joints and tendons in all parts of the body. Musculoskeletal grievance can be constant or intermittent, that usually persists for a long period of time and is not always attributed to a specific cause. The data of Health Research and Development Agency in 2013 showed prevalence of workers suffering from Musculoskeletal Disorders in Indonesia of 11.9% and workers reporting musculoskeletal grievance of 24.7%. Musculoskeletal grievance caused by many factors. According to Graveling, musculoskeletal grievance caused by repetition, posture, force, and duration. Research conducted by Tjahayuningtyas showed that musculoskeletal grievance caused by age, exercise habit, posture, job tenure, body mass index and workload.

Study related to musculoskeletal grievance in the batik workers have been carried out in Indonesia. Research conducted by Santosa and Ariska in 2018 showed that risk factors affecting musculoskeletal grievance are type of work, age, work procedure, working posture, gender and working tenure. The study of Savitri, Hardian, and Sumekar in 2015 showed that inappropriate working posture when batik making caused musculoskeletal grievance. The study of Ramadhiani, Widjasena, and Jayanti in the batik workers in Kampoeng Batik Laweyan Surakarta showed that working duration and shoulder’s angle while extending are risk factors of musculoskeletal grievance. The research conducted by Isnaini, Bagyono, and Hendrarini in women lighters in the Village of Jarum Klaten showed that musculoskeletal grievance caused by working duration.

Batik Madura is one of informal industries engaged in the manufacture of batik cloth that develops in Bangkalan Regency. In the region, Tanjung Bumi Sub-District is among the largest producer and supplier of batik in Madura. The production activities in Batik Madura composed by several steps, such as the washing of plain mori cloth (Ketel), the drawing of the outlines of batik pattern (Rengreng), the drawing of small designs (Kuri), the application of wax using canting (Essean), the covering of cloth portion in which no designs are drawn (Nebbeng), the process of dyeing, performed twice, and removal or dissolution of wax using boiling water (Lorot). This study aims to determine the relationship between working tenure and working posture with musculoskeletal grievance in Batik Madura workers.

Materials and Methods

This study was an observational study with a cross-sectional design. This study was conducted on Batik Madura’s home industry, Tanjung Bumi Sub-District, Bangkalan Regency, Madura, Indonesia in January 2020. The population of this study was all Batik Madura workers as much as 81 people. The sampling technique in this study used with proportional stratified random sampling. Sample used in this study was all batik workers in Batik Madura’s home industry as much as 61 people.

Measurement of working tenure was carried out by filling out a research questionnaire and interview. Before asking respondents about working tenure, respondents were given an explanation about research implementation and respondents asked to fill out an informed consent if they agreed to take part in the research. Working tenure categorized into beginning, moderate, and long categories. Measurement of the working posture was carried out by observation when the workers during their work activities. The observation was performed by taking pictures of the workers’ working postures during batik production which were then analyzed using the Rapid Entire Body Assessment (REBA). The working posture categorized into low, moderate, high, and very high categories. Next, the respondents were asked to fill a Nordic Body Map (NBM) questionnaire containing 28 limbs presenting with musculoskeletal grievance by inserting a check mark on one of MSD grievance choices namely no pain, mildly painful, painful, and very painful.
according to their individual condition.

The data analysis technique in this study was univariate and bivariate analysis. Univariate analysis was conducted by determine frequency distribution for each variable, such as working tenure, working posture and musculoskeletal grievance. The bivariate analysis was carried out to analyze the relationship between working tenure and working posture with musculoskeletal grievance using the Spearman correlation test with $\alpha = 0.05$.

**Results and Discussion**

**Working Tenure**

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>22</td>
<td>36.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>41.0</td>
</tr>
<tr>
<td>Long</td>
<td>14</td>
<td>23.0</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The results of working tenure were categorized in to 3 categories, namely beginning, moderate and long. Table 1 showed that 25 respondents (41%) was in beginning category, 22 respondents (36%) was in moderate category and 14 respondents (23%) was in long category.

Working tenure is one of musculoskeletal grievance risk factors. Working tenure can cause musculoskeletal grievance because working tenure will cause fatigue, monotonous, and repetitive work activities, which are the main cause of musculoskeletal grievance\textsuperscript{15}.

**Working Posture**

The assessment of working posture on 61 Batik Madura workers was performed by documenting working posture of the Batik Madura workers during working activities in the form of photos and videos. Then, the documentation results analyzed with the Rapid Entire Body Assessment (REBA) method. REBA way was divided into two clusters, A and B. Cluster A REBA analyzed body parts, namely neck, legs and trunk while Cluster B REBA analyzed body parts, namely forearms, upper arms, and wrists.

The results of working posture assessment with the Rapid Entire Body Assessment (REBA) method were categorized into 4 categories, namely low, moderate, high, and very high categories. Table 2 showed that 27 respondents (44.3%) had working posture in the high category risk level, 20 respondents (32.8%) were in the very high category risk level, 14 respondents (23%) were in the moderate category risk level, and no one respondents in the low category of working posture.
Table 2. Distribution of Working Posture in Batik Madura Workers

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>14</td>
<td>23.0</td>
</tr>
<tr>
<td>High</td>
<td>27</td>
<td>44.3</td>
</tr>
<tr>
<td>Very high</td>
<td>20</td>
<td>32.8</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The rigid working posture causes the position of body parts move away from the natural positions. This condition lead the workers have a risk of musculoskeletal grievance

Musculoskeletal Grievance

The assessment of musculoskeletal grievance perceived by the 61 Batik Madura workers was performed by the way of interviews and filling the Nordic Body Map (NBM) questionnaire containing 28 body muscles were felt pain by the workers. The musculoskeletal grievance was categorized into low, moderate, high, and very high categories. Table 3 showed that 25 respondents (41%) in the high category, 20 respondents (32.8%) in the very high category, 16 respondents (26.2%) in the moderate category, and no one respondents in the low category of musculoskeletal grievance.

Table 3. Distribution of Musculoskeletal Grievance in Batik Madura Workers

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>16</td>
<td>26.2</td>
</tr>
<tr>
<td>High</td>
<td>25</td>
<td>41.0</td>
</tr>
<tr>
<td>Very high</td>
<td>20</td>
<td>32.8</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The result of musculoskeletal grievance in 61 Batik Madura workers measured on 61 Batik Madura workers using Nordic Body Map questionnaire showed that 28 respondents (45.9%) felt very pain in the spine. 23 respondents (37.7%) felt pain in the waist. The most rather pain felt by 23 respondents (37.7%) in the left leg and 21 respondents (34.43%) felt less pain in the left elbow. The results of musculoskeletal complaint show Table 4 below.
Table 4. Distribution of Musculoskeletal Grievance Location in Batik Madura Workers

<table>
<thead>
<tr>
<th>Organ</th>
<th>No Pain</th>
<th>Mildly Painful</th>
<th>Painful</th>
<th>Very Painful</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Upper neck</td>
<td>15</td>
<td>24.59</td>
<td>12</td>
<td>19.67</td>
<td>20</td>
</tr>
<tr>
<td>Lower neck</td>
<td>11</td>
<td>18.03</td>
<td>12</td>
<td>19.67</td>
<td>16</td>
</tr>
<tr>
<td>Left shoulder</td>
<td>12</td>
<td>19.67</td>
<td>16</td>
<td>26.23</td>
<td>16</td>
</tr>
<tr>
<td>Right shoulder</td>
<td>11</td>
<td>18.03</td>
<td>16</td>
<td>26.23</td>
<td>16</td>
</tr>
<tr>
<td>Left upper arm</td>
<td>12</td>
<td>19.67</td>
<td>16</td>
<td>26.23</td>
<td>17</td>
</tr>
<tr>
<td>Spine</td>
<td>8</td>
<td>13.11</td>
<td>9</td>
<td>14.75</td>
<td>16</td>
</tr>
<tr>
<td>Right upper arm</td>
<td>8</td>
<td>13.11</td>
<td>9</td>
<td>14.75</td>
<td>19</td>
</tr>
<tr>
<td>Waist</td>
<td>7</td>
<td>11.48</td>
<td>8</td>
<td>13.11</td>
<td>23</td>
</tr>
<tr>
<td>Buttock</td>
<td>9</td>
<td>14.75</td>
<td>7</td>
<td>11.48</td>
<td>20</td>
</tr>
<tr>
<td>Buttom</td>
<td>8</td>
<td>13.11</td>
<td>10</td>
<td>16.39</td>
<td>17</td>
</tr>
<tr>
<td>Left elbow</td>
<td>21</td>
<td>34.43</td>
<td>22</td>
<td>36.07</td>
<td>9</td>
</tr>
<tr>
<td>Right elbow</td>
<td>17</td>
<td>27.87</td>
<td>13</td>
<td>21.31</td>
<td>17</td>
</tr>
<tr>
<td>Left lower arm</td>
<td>9</td>
<td>14.75</td>
<td>17</td>
<td>27.87</td>
<td>17</td>
</tr>
<tr>
<td>Right lower arm</td>
<td>9</td>
<td>14.75</td>
<td>17</td>
<td>27.87</td>
<td>19</td>
</tr>
<tr>
<td>Left wrist</td>
<td>10</td>
<td>16.39</td>
<td>11</td>
<td>18.03</td>
<td>20</td>
</tr>
<tr>
<td>Right wrist</td>
<td>10</td>
<td>16.39</td>
<td>12</td>
<td>19.67</td>
<td>18</td>
</tr>
<tr>
<td>Left hand</td>
<td>20</td>
<td>32.79</td>
<td>17</td>
<td>27.87</td>
<td>13</td>
</tr>
<tr>
<td>Right hand</td>
<td>18</td>
<td>29.51</td>
<td>20</td>
<td>32.79</td>
<td>8</td>
</tr>
<tr>
<td>Left thigh</td>
<td>14</td>
<td>22.95</td>
<td>10</td>
<td>16.39</td>
<td>10</td>
</tr>
<tr>
<td>Right thigh</td>
<td>15</td>
<td>24.59</td>
<td>12</td>
<td>19.67</td>
<td>10</td>
</tr>
<tr>
<td>Left knee</td>
<td>13</td>
<td>21.31</td>
<td>13</td>
<td>21.31</td>
<td>18</td>
</tr>
<tr>
<td>Right knee</td>
<td>16</td>
<td>26.23</td>
<td>11</td>
<td>18.03</td>
<td>14</td>
</tr>
<tr>
<td>Left calf</td>
<td>9</td>
<td>14.75</td>
<td>14</td>
<td>22.95</td>
<td>20</td>
</tr>
<tr>
<td>Right calf</td>
<td>10</td>
<td>16.39</td>
<td>12</td>
<td>19.67</td>
<td>17</td>
</tr>
<tr>
<td>Left ankle</td>
<td>15</td>
<td>24.59</td>
<td>19</td>
<td>31.15</td>
<td>12</td>
</tr>
<tr>
<td>Right ankle</td>
<td>15</td>
<td>24.59</td>
<td>14</td>
<td>22.95</td>
<td>15</td>
</tr>
<tr>
<td>Left leg</td>
<td>17</td>
<td>27.87</td>
<td>23</td>
<td>37.70</td>
<td>14</td>
</tr>
<tr>
<td>Right leg</td>
<td>19</td>
<td>31.15</td>
<td>11</td>
<td>18.03</td>
<td>20</td>
</tr>
</tbody>
</table>

Relationship Between Working Tenure with Musculoskeletal Grievance
Table 5. Distribution of Relationship Between Working Tenure with Musculoskeletal Grievance

<table>
<thead>
<tr>
<th>Working Tenure</th>
<th>Musculoskeletal Grievance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Beginning</td>
<td>4</td>
<td>18.2</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td>Long</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>26.2</td>
</tr>
</tbody>
</table>

p-value = 0.837

* Significant at p-value < 0.05

The statistical analyze using Spearman Correlation produced p-value of 0.837. It means that there is no relationship between working tenure with musculoskeletal grievance. This study were in line with the study by M.A, Sabilu, and Pratiwi in 2016 showed that there was no relationship between working tenure with musculoskeletal grievance. The study of M.A, Sabilu, and Pratiwi in 2016 showed that working tenure is a related factor with length of time of someone has worked in a industry. Related to this, musculoskeletal grievance is chronic disease that requires a long time to develop and manifest. Someone has a long working tenure so someone exposed to these risk factors for musculoskeletal disorders then greater the risk for developing musculoskeletal grievance. There is no relationship between working tenure with musculoskeletal grievance on Batik Madura workers because most of the workers are recruited by Batik Madura’s owners during their working period in the beginning category, so that the musculoskeletal grievance felt by the workers are not based on the working tenure the worker has.

Relationship Between Working Posture with Musculoskeletal Grievance

Table 6. Distribution of Relationship Between Working Posture with Musculoskeletal Grievance

<table>
<thead>
<tr>
<th>Working Posture</th>
<th>Musculoskeletal Grievance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Moderate</td>
<td>11</td>
<td>78.6</td>
</tr>
<tr>
<td>High</td>
<td>5</td>
<td>18.5</td>
</tr>
<tr>
<td>Very High</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>26.2</td>
</tr>
</tbody>
</table>

p-value = 0.000

* Significant at p-value < 0.05
The statistical test using the Spearman Correlation produced a p-value of 0.000. It means that there was a relationship between working posture with musculoskeletal grievance. Static working posture during working activities cause blockage of blood flow in the body so the body's organs suffer from deficit of oxygen and glucose in the blood. This condition forces body to generate a metabolism byproduct in the form of lactic acid that gives rise to soreness when it builds up. This study were in line with Saputro, Mulyono, and Puspikawati in 2018, the study showed that there was a correlation between working posture and musculoskeletal grievance in batik workers in Virdes Batik Collection. Similarly, Santosa and Ariska also found a correlation between working posture and musculoskeletal grievance in batik artisans with a p-value of 0.001. Unergonomic working posture make up the position of the body parts move away from natural positions. This posture shows strong evidence as a risk factor which can lead to musculoskeletal grievance.

**Conclusion**

This study draws conclusions that the majority of Batik Madura workers have a working tenure in the beginning category, working posture in high category, and musculoskeletal grievance in high category. There is no relationship between working tenure with musculoskeletal grievance in the Batik Madura workers. There is a relationship between working posture with musculoskeletal grievance in the Batik Madura workers.

To avoid musculoskeletal grievance in the Batik Madura workers, workers are advised to break for 20 minutes when working by moving head, leg and body. The owners of Batik Madura advised to do a redesign to workplace starting by providing chair with back support and armrests as well as *gawangan* (a bamboo frame over which the cloth to be waxed is draped).

**Acknowledgments:** We would like to say thank you to all parties that are involved in this study, especially workers and the owners of Batik Madura who have been willing to become the respondents in this research.

**Ethical Clearance:** This study was approved by Ethic Committee in Faculty of Dental Medicine, Universitas Airlangga, Indonesia with registration number 325/HRECC.FODM/VII/2020.

**Source of Funding:** The source of funding in this study from author’s personal funds.

**Conflict of Interest:** Nil

**References**

Effectiveness of Transport Guidelines on Intra Hospital Transport Practices of Nurses and Occurrence of Mishaps among Critically ill Patients

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Abstract

Background: Intrahospital transport means transporting ill patients for diagnostic and therapeutic procedures within the hospital. Implementation of intrahospital transfer demands a well organized meticulous planning and prompt application of well designed plan.

Objectives: To compare intrahospital transportation practices and occurrence of mishaps during intrahospital transport before and after administration of hospital transport guidelines and to determine the association with situational variables (level of floors and distance of destination)

Methods: Quasi experimental one group pre test post test design was used in the study by event sampling. 55 pre test transport and 62 post test events were selected. IHT guidelines was given to nurses working in operation theater by using demonstration cum lecture technique, one on one teaching provided to individual nurse consecutively. Observational technique was used for data collection. Result showed that the mean percentage of intrahospital practice were higher (89.37%) then pre test (40.03) with a mean difference of 49.33. Computed t value (t=37.46,p=0.000) was statistically significant. Chi square value of occurrence of mishaps regarding intrahospital transport is ($X^2=53.9$,p=0.001) statistically significant. F value between transportation practices with distance from OT were (f=1.68,p=0.16) and Computed t value practices with level of floor were (t=1.60,p=0.39) statistically not significant at 0.05 level of significance. Conclusion Intrahospital transportation guidelines are effective in reducing mishaps and increases transportation practices.

Key Words: Intra hospital transport practices, mishaps, transport guidelines

Introduction

Intrahospital transport (IHT) means transporting ill patients for diagnostic and therapeutic procedures with in the hospital. An assured quality of IHT can be maintained through the ground formula of a significant quality of intrahospital transfer which provides degree of care before, during and after transportation. A close intermediate and continuing communication are required for smooth transfer till the destination department for transfer1. The care of acutely ill patients includes constant monitoring and adequate intervention to protect client from harms or prevent mishaps, it is an integral responsibility of nurses2. Several studies have shown that transporting critically ill patients can lead to alteration in their physiological status and they are at acute risk of increased morbidity and mortality3. Researcher could only locate one study related to effect of transport guidelines on mishaps and none of the studies on transport practices moreover located studies are concerning intrahospital transport focused on I.C.U and emergency rather than Operation theatre’s (O.T) patients, also most of the studies are not recent. However OT patients are under anesthesia and have limited physiological reserves. Hence effect of intervention on reducing the mishaps for OT patients has received little attention. Therefore present study has been conducted to improve the IHT practices and reduction of mishaps in O.T patients (patients who had undergone surgery and are being shifted to destination department from OT’s recovery room). The purpose of
the study was to evaluate the effectiveness of transport guidelines on Intra Hospital Transport Practices of Nurses and Occurrence of Mishaps among Critically ill Patients which miscellaneous causes 64% was highest including oxygen probes(27.33%), ECG leads displacement (19.42), oxygen saturation was dropped in 10.79 patients, 3.59% and 4.31 were reported of altered mental status and arrhythmia respectively, 3 patients out of 64 have been terminated from the transport due to serious adverse effects.

Method and Material

Quantitative research approach and Quasi experimental one group pre test post test study design was used to assess the effectiveness of transport guidelines on IHT practices of nurses and occurrence of mishaps among critically ill patients, Rosswrum and larrabee conceptual frame work was used and figure 1 shows schematic representation of research. Study was conducted in 950 bedded multispecialty teaching hospital which consist of 8 operating theaters consist of three general OT, one neurosurgery OT, one gynae surgery OT, one Cardio thorax OT, one eye OT, one ENT OT and 3 minor OT. Duration of data collection was three months from December 2015 to January 2016. Sample size was calculated using power analysis with 5% significance and 80% power, standard deviation of the transport being studied (σ=7.74) based on previously published study, where as the size of the effect, mean difference (d=3.06) was calculated from annual census of surgery occurred at OT. Event sampling was used to observe the events of transportation and total enumerative sampling method was used to select nurses for checking acceptability of transport guidelines. 55 transport events before administration of transport guidelines 62 transport events after administration of transport guidelines.

Data was collected using three tools for the study tool one: Observational Checklist for Assessment of Intra Hospital Transport Practice first section consist of situational variables which include 2 items place of transport :distance from OT to designated ward (measurement was taken from university architectural department) and level of floors. Section 2 consist of observation checklist for assessment of intra hospital transport practices it comprises of three areas before, during and after transportation with total items 17,10,8 and weightage 48.5,28.5,22.85 respectively, also the level of practice in terms of intrahospital transport were very good (>85%), good (66-85%), average (51-65%), below average (<50%).

Second tool was checklist for assessing occurrence of mishaps, it contains 13 items disconnection of urinary catheter, backflow of urine, disconnection of surgical, displacement of oxygen supply , displacement of saturation probe etc with individual score range of checklist (0-13).Third tool was acceptability scale it was prepared to assess the acceptability of transport guidelines among nurses it contains 5 semantic differential scale consist of 7 items easy, valuable, time saving, practical, beneficial, effective on reducing mishaps , 5 point scale has rating from 1-7, individual score ranges from 7-35.

To ensure content validity of the tool it was submitted to 7 experts from nursing field of medical surgical department and child health nursing department also 2 experts from medical ICU, and surgical ICU were asked to mark one response out of 3 responses like “fully met” “mostly met” “to some extent” and requested to give suggestions and remarks, all three tools were validated after incorporating the suggestions and modifications given by experts, scale content validity index by averaging item content validity was 0.80. Consensus measure of inter rater reliability for observational coding by asking experts from neurosurgery ,emergency department and researcher to mark each tool items against response of dichotomous “yes” or “no” , later cohen’s kappa coefficient was 0.70 for transport practices and 0.72 for occurrence of mishaps was established . Development and validation of guidelines was modified with contribution of health professional from various area, later organization of content, language, practicability and feasibility was established in pilot study, all corrections were incorporated in final intervention. Occurrence of events of transportation of patients who has surgery under general/spinal anesthesia from OT (general, gynae, ortho, ENT, neuro ) to destination wards (recovery ICU, surgical ICU, surgery ward A,B,C) under presence of researcher, also staff nurses who were available during the time of data collection were included into the study, where as events of transportation occurred in absence of researcher or patient who had surgery under local anesthesia or events from pediatric OT and staff nurses
who were not available at the time of data collection were excluded from the study.

Ethical approval from university communication of decision of ethics committee (IEC) project no. 371 was obtained from medical superintendent, written informed consent was obtained from study subject after explaining about the research project and assurance of confidentiality was given. In order to develop rapport, introduction to self and study was given to the nurses working in OT. After obtaining informed consent, from day one to day 14 before intervention 55 events of intra transport practices and occurrence of mishaps were assessed, from day 15 to 19 a transport guidelines through group teaching (45min) was administered including demonstration of two transport events and return demonstration of two events by individual staff nurse. From day 20 to 34 after administration of guidelines 62 events were assessed.

For statistical analysis, using SPSS version 17 both descriptive and inferential statistics were used to compare continuous variable student’s t test was used and chi square to compare percentage p<0.05 was considered statistically significant. Data were expressed as mean% ± standard deviation (SD) the association between mishaps and situational variables were also studied using ANOVA (in the present study the dependent variable (D.V) i.e. intrahospital practices were assessed through checklist, each correct transport practices was scored 01 and incorrect 00. And a total practices score was obtained, therefore to seek association of D.V i.e. IHT practices with situational variables (independent variable (I.V)), ANOVA was used. For ANOVA to be applied D.V should be in interval/ratio scale and I.V should be in nominal/ categorical scale, as practices were measured interval scale by assigning score to them, therefore, D.V in the study was meeting eligibility of ANOVA).

**Result**

Analysis and interpretation of data were based on the objectives and hypothesis to be tested. The objectives of the study were to compare intrahospital transport practices before and after administration of transport guidelines. To compare occurrence of mishaps during intrahospital transport before and after administration of hospital transport guidelines. To determine the association of transport practices with situational variables. To determine the acceptability of transport guidelines among nurses. The following hypotheses were tested at 0.05 level of significance:

H1. There will be significant difference in the mean practice score regarding intrahospital transport before and after administration of transport guidelines.

H2. There will be significant difference in the occurrence of mishaps during intrahospital transport before and after administration of transport guidelines.

H3. There will be significant association of transport practices with situational variables.

In null hypothesis there is no relationship between variables and that any observed relationship are results of chance or sample fluctuations.

In terms of comparison of situational variables, in pre test the majority of events were from OT to ICU(34.5%) followed by surgery B (18.2%) surgery A (18.2%), surgery C(16.4%) and RICU(12.7%). 57.2% events of transport occurred on other floor than the OT, about 47.3% events of transport occurred on same floor at OT, whereas in post test majority of events of transport were from OT to ICU i.e. 33.9% surgery B(21%) surgery A (17.7%) surgery C (16.1%), RICU (11.3%). 54.8% events of transport occurred on other floor then the OT and 45.2% occurred on same floor. Chi square was applied to compare pre test and post events with respect to place of transport ($\chi^2=0.09$, $p=0.24$) level of floor ($\chi^2=2.00$, $p=0.50$) compute chi value was found to be not significant at 0.05 level of significance hence events of transportation were homogenous and comparable in terms of situational variables. Bar graph (figure: 2) shows frequency percentage distribution of transport events and concludes that level of practices were improved.

In pre test range of IHT practice score was 21.87 to 70.58 with mean % and SD 40.03±9.11, median 37 whereas in post test range was 78.12 to 93.75 with mean% and SD 89.37±4.67, median 90, (Table no. 1) computed ‘t’ value ($t=37.46$, $p=0.001$) was found to be statistically significant at 0.05 level of significance which shows that mean difference obtained was true difference and not by chance, Hence intra hospital guideline were
effective in improving IHT practices also in area wise i.e. before, during and after transport (Table no.2) was found to be highly significant at 0.05 level of significance in each area which shows true mean difference and not by chance hence improved IHT practices, therefore H₁ accepted, while assessing effectiveness of transport guidelines in terms of occurrence of mishaps (Table no.3) chi square value regarding intrahospital transport was statistically significant at 0.05 level of significance with disconnection of urinary catheters ($\chi^2 = 2.72$, $p=0.03$) backflow of urine ($\chi^2 = 40.01$, $p=0.001$), disconnection of surgical drain ($\chi^2 = 21.59$, $p=0.001$), showing significance difference obtained of occurrence of mishaps whereas disconnection of oxygen supply, dislodgment of oxygen probe, disconnection of intravenous line ($\chi^2 = 0.09$, $p=0.75$) in pre test 94 times out of 473 (total applicable mishaps) i.e. 19.87% and 25 times out of 542 (4.77%) mishaps occurred in post test and chi square ($\chi^2 = 53.94$, $p=0.001$) was found to be statically significant at 0.05 level of significance. Table no. 4 shows t value was significant and inferred that significant difference in occurrence of mishaps in before and after administration of IHT guidelines was effective on reducing occurrence of mishaps, (in this table all values are calculated in % as the no. of practices were varying, denominators are different in pre and post test, level of ) hence hypothesis H2 accepted. Also, level of acceptability (n=22) regarding IHT guidelines in figure 3 concludes the guidelines were acceptable by nurses in terms of easy, valuable, time, saving, acceptable and practical. In terms of association of transport practices with situational variables (Table no. 5), findings reveals that the transportation practices was not associated with place of transport and level of floor were independent with respond to situational variables hence research hypothesis H₃ was rejected.

\[
\begin{array}{ccc}
O_{P_1,M_1} & X & O_{P_2,M_2} \\
\end{array}
\]

Fig:1 Schematic representation of study design

$O_{P_1} =$ Intrahospital transport practices before administration of transport guidelines  

$M_1 =$ Occurrences of mishaps during intrahospital transport before administration of transport guidelines.  

$X =$ Administration of Transport Guidelines

$O_{P_2} =$ Intrahospital transport practices after administration of transport guidelines

$M_2 =$ Occurrences of mishaps during intrahospital transport after administration of transport guidelines

Figure: 2 Transport events in terms of intra hospital transport practices
N=22

Figure 3: Acceptability of IHT guidelines by nurses

Table 1: Mean, Mean Difference, Standard Error of Mean Difference and ‘t’ value of Transport Event’s Practice Scores

<table>
<thead>
<tr>
<th>Practice Score</th>
<th>Mean</th>
<th>MD</th>
<th>SEMD</th>
<th>df</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre test</td>
<td>40.03</td>
<td>49.33</td>
<td>1.31</td>
<td>115</td>
<td>37.46*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Post test</td>
<td>89.37</td>
<td>1.60</td>
<td>15.16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$t(115)=1.98$ *significant (p≤ 0.05)

Table 2: Area wise Mean, Mean deviation and standard error of mean deviation and t value of transport event’s practice score in terms of percentage of before and after administration of intrahospital transport guidelines.

Pretest n=55
Post test n=62

<table>
<thead>
<tr>
<th>Area</th>
<th>Mean</th>
<th>MD</th>
<th>SEMD</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before transport</td>
<td>67.95</td>
<td>24.27</td>
<td>1.60</td>
<td>15.16</td>
<td>0.00*</td>
</tr>
<tr>
<td>Pre test</td>
<td>92.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post test</td>
<td>26</td>
<td>60.30</td>
<td>1.72</td>
<td>34.93</td>
<td>0.00*</td>
</tr>
<tr>
<td>During transport</td>
<td>86.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>26</td>
<td>60.30</td>
<td>1.72</td>
<td>34.93</td>
<td>0.00*</td>
</tr>
<tr>
<td>Post test</td>
<td>87.50</td>
<td>84.31</td>
<td>1.98</td>
<td>42.49</td>
<td>0.00*</td>
</tr>
<tr>
<td>After transport</td>
<td>3.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>87.50</td>
<td>84.31</td>
<td>1.98</td>
<td>42.49</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

$t(115)=1.98$ *significant (p≤ 0.05)
Table no: 3: Chi Value of Occurrence of Mishaps Regarding Intra Hospital Transport

<table>
<thead>
<tr>
<th>Mishaps</th>
<th>Pre test (n=55)</th>
<th>Post test(n=62)</th>
<th>df</th>
<th>chi</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Applicable</td>
<td>Not occurred</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>occurred</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disconnection of oxygen supply</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Displacement of O2 saturation probe</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Disconnection of intravenous line</td>
<td>55</td>
<td>3</td>
<td>52</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>Obstruction of intravenous line</td>
<td>55</td>
<td>6</td>
<td>49</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Backflow of blood in intravenous lines</td>
<td>55</td>
<td>7</td>
<td>48</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Disconnection of central venous catheter line</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Disconnection of urinary catheter</td>
<td>55</td>
<td>4</td>
<td>51</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>Backflow of urine</td>
<td>55</td>
<td>32</td>
<td>23</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Disconnection/obstruction of surgical drains</td>
<td>55</td>
<td>20</td>
<td>35</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>Accidental falling of patient from stretcher</td>
<td>55</td>
<td>2</td>
<td>53</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Falling of monitor and devices from stretcher</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Pricks/injury from uneven surface of stretcher</td>
<td>55</td>
<td>6</td>
<td>49</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Dislocation of oral airway</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>473</td>
<td>94</td>
<td>379</td>
<td>524</td>
<td>25</td>
</tr>
</tbody>
</table>

TABLE : 4 Mean, Mean Difference, Standard Error of Mean Difference and ‘t’ value of Occurrence of Mishaps Regarding Intra Hospital Transport

<table>
<thead>
<tr>
<th>Practice score</th>
<th>Mean</th>
<th>MD</th>
<th>SEMD</th>
<th>df</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre test (n-55)</td>
<td>19.85</td>
<td>14.89</td>
<td>2.26</td>
<td>115</td>
<td>6.57*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Post test (n-62)</td>
<td>4.96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$t (115)= 1.98$   
*significant (p≤ 0.05)
Table: 5 ANOVA / t test Value Showing Association of Transport Practice With situational Variables

<table>
<thead>
<tr>
<th>Situational variables</th>
<th>Mean score</th>
<th>F/t value</th>
<th>df1/df2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place of transport (distance from OT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RICU (78.9m)</td>
<td>85.83</td>
<td>1.682</td>
<td>4/57</td>
<td>0.167NS</td>
</tr>
<tr>
<td>ICU (97.5m)</td>
<td>89.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery C (125.4m)</td>
<td>90.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery B (164.4m)</td>
<td>89.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery A (203.4m)</td>
<td>91.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of floor t</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same floor</td>
<td>88.34</td>
<td>1.601</td>
<td>60</td>
<td>0.393NS</td>
</tr>
<tr>
<td>Other floor</td>
<td>90.22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In respect to aim of the study the intrahospital guidelines were effective in reducing mishaps and increase in level of practice. In the present study pre test depict, the level of practice regarding IHT was below average (79%) in the majority of events whereas in post test, it was very good (79%) in the majority of events followed by good (21%) practices. Also chi value showing comparison between occurrence of mishaps in pre and post test depicts that after administration of guidelines occurrence of mishaps decreased in post (94 to 25, p-0.001) the findings of the study was consistent with the study conducted by H.K Choi et al\(^5\), in which unexpected events decreased significantly (36% -22.1%, p-0.001) and after administration of guidelines disconnection of oxygen supply has increased in (50% to 69.23%,p-0.567) findings of the study was inconsistent with the study conducted by Choi et al, in which line disconnected for oxygen supply decreased (10.1% -2.6%,p-0.19) was also observed that transportation practices were independent of distance of transport’s designation, (p-0.167) finding of the study were consistent with the study conducted by Despoina G.A, & Brokalaki H.\(^6\), that distance from destination did not appear to affect the interruption frequency of patient’s treatment(0.190). Another finding the displacement of oxygen probe in pre test events was 50% whereas in a study conducted by Rao S, Muktule D, Taggu A, & Venkategowda P\(^4\), shows 27.83% of oxygen probe unexpected events has occurred. In present study, out of 55 pre test events 18.18% of doctors, 5.45% of nurses and 100% attendants were present while transporting patients(data collected from one of the item in IHT practices tool), a similar findings shown by Silmara M\(^7\) in which 77.3% of the team were composed of physician, nurse and nurse technician. 50% of oxygen desaturation occurs in 55 pre test events, where as in study done by Kue R, Brown P, Ness C, & Scheulen J\(^8\) reported 8.8% of oxygen desaturation occurred during intra hospital transport. In present study pre test events, 98.19% had poor communication whereas in a study conducted by Beckmann U. Et.al\(^9\), 61% of the incidence reflected to staff management issue including poor communication and inadequate monitoring. The study was confined to small group and lack control group also hawthorn effect was not controlled in the study and extraneous variables like age and severity of illness of the patients were not assessed.

Recommendation

Based on findings of study a replica on large sample can be done to make generalization, randomized control
Trial cab be conducted also experimental study can be done to evaluate the knowledge and attitude of nurse regarding use of transport guidelines. A standardized transportation, further research can be conducted to explore physiological changes and emotional experiences of patients.

**Conclusion**

Intrahospital transport guidelines were effective in reducing mishaps and increases transportation practices. Common mishaps identified were backflow of urine, disconnection of I.V lines, and there was no association found in between situational variables and intrahospital transport guidelines.

**References**

Knowledge of Women about the Early Detection Methods of Cervical Cancer in Baghdad City

Iman A Jaber
Instructor, Baghdad College of Medical Sciences

Abstract

Background: Cervical Cancer is considering a public health problem, leading cause of mortality and morbidity among women.

Objective: To assess women’s knowledge regarding cervical cancer and early detection methods

Methodology: A descriptive study was conducted, included (200) women from different levels of education who work in the institute, from 5th November 2018 to 30 April 2019, by using interview technique and self-reporting technique. A non-probability (purposive) sample of (200) women. The questionnaire was used for data collection. The validity was estimated through a penal of experts related to the field of study, and so the reliability was evaluated through a pilot study conduct included (10) women (except from the original sample). Data is analyst through the implementation of descriptive and inferential statistical analysis.

Results: The results of this study show that the knowledge of the study sample was low regarding early detection methods, prevention treatment and there was statistical significant relationship between level of knowledge, and some variables

Recommendations: It was recommended that increase coverage in cervical screening programs from Iraq ministry of health to encourage women in Iraq. In addition, training program should supply in educational institute

Key words: Cervical Cancer, Knowledge Pap Smear and Women

Introduction

Globally, they found cervical cancer is the fourth most frequent cancer among women (1).

Cervical Cancer was a public health problem and a leading cause of mortality and morbidity among women (2,3) and it is the fourth most frequently diagnosed cancer with an estimated 527,600 new cases in 2012 worldwide. It is the fourth leading cause of cancer death with 265,700 deaths among women worldwide in 2012 (4,5) and so it was the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 appear 6.6% of all female cancers. Approximately 90% of deaths from cervical cancer occurred in developing countries. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, and effective screening and treatment programmers. There are currently vaccines that protect against common cancer that cause types of human papilloma virus and can significantly reduce the risk of cervical cancer (1).

There are contributory factors that make women vulnerable to develop cervical cancer, viral infections (HPV, HIV, and HSV), multiparty, early initiation of sexual activity, multiple sex partners, smoking, low socioeconomic status, diet low in antioxidants, poor hygiene, long-term use of oral contraceptives and immune suppression conditions (6).

The incidence of cervical cancer in Iraq it relatively low, as in most other Islamic countries, yet most of the cases usually present in advanced stages with poor prospects of cure. Earlier studies have illustrated un negligible rates of CIN lesions among Iraqi patients.
complaining of gynecological problems (221) Incidence Rate 1.20 /100,000P (7).

Common risk factors include early age at first intercourse, having multiple sexual partners and a weak immune system. Research evidence has shown that Human Papilloma virus (HPV) is the most important etiologic agent in the vast majority of cases and the cause of 99.7% of cervical cancer cases, which is among the most frequent cancers in women (8,9).

Aim of study: To assess women’s knowledge and attitudes regarding cervical cancer and early detection methods of cervical cancer for the teacher and employer who were working in Technical Medicine Institute, Baghdad

Methodology
A descriptive study was included (200) women from different levels of education who work in Technical Medicine Institute, Baghdad Through using the assessment approach for the period from 5th November 2018 to 30 April 2019. The questionnaire was used for data collection. The validity was estimated through a penal of experts related to the field of study, and so us the reliability was evaluated through a pilot study included 10 women. The questionnaire form was consisted of (3) main part. The data were collected by using interview method and self-report techniques with study participants after obtaining permission from each of them according to the inclusion criteria.

Reliability of the questionnaire was estimated through the use of Alpha Cronbach for the test-retest approach, descriptive statistics (frequency, percentage Cum. Percent, Mean of score (M.S.), and Relative Sufficiency (R.S.)) and inferential statistics (Alpha Cronbach, Reliability Coefficient, Chi Square).The items of women documentation were rated on two level know, and don’t know, and scored as , 2 and 1, respectively ). Mean of score Low (1-1.49), Moderate (1.5 – 1.75), and High (1.76 –2]

Results of the Study
Table (1): Assessment of women’ knowledge related to the early detection

<table>
<thead>
<tr>
<th>N</th>
<th>Items</th>
<th>Know</th>
<th>I do not</th>
<th>M S</th>
<th>R S</th>
<th>Asses.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Cervical cancer is: Cervical cancer is a cancer that affects the cervix</td>
<td>74</td>
<td>126</td>
<td>1.37</td>
<td>68.5</td>
<td>L</td>
</tr>
<tr>
<td>1.2</td>
<td>Cervical cancer usually develops very slowly</td>
<td>80</td>
<td>120</td>
<td>1.4</td>
<td>70.0</td>
<td>L</td>
</tr>
<tr>
<td>1.3</td>
<td>Cancer changes can take years to develop into cervical cancer</td>
<td>77</td>
<td>123</td>
<td>1.38</td>
<td>69.0</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>Risk factors for cervical cancer:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>The majority of cervical cancers are caused by HPV,</td>
<td>78</td>
<td>122</td>
<td>1.39</td>
<td>69.5</td>
<td>L</td>
</tr>
<tr>
<td>2.2</td>
<td>Early marriage (early sex).</td>
<td>123</td>
<td>77</td>
<td>1.6</td>
<td>80.75</td>
<td>M</td>
</tr>
<tr>
<td>2.3</td>
<td>Smoking</td>
<td>149</td>
<td>51</td>
<td>1.75</td>
<td>87.25</td>
<td>M</td>
</tr>
<tr>
<td>2.4</td>
<td>HIV infection (the virus that causes AIDS)</td>
<td>52</td>
<td>148</td>
<td>1.26</td>
<td>63</td>
<td>L</td>
</tr>
<tr>
<td>2.5</td>
<td>Use pills for a long time (five years or more).</td>
<td>100</td>
<td>100</td>
<td>1.5</td>
<td>75</td>
<td>M</td>
</tr>
<tr>
<td>2.6</td>
<td>Multiple births.</td>
<td>112</td>
<td>88</td>
<td>1.65</td>
<td>78</td>
<td>M</td>
</tr>
<tr>
<td>2.7</td>
<td>Couples who engage in high-risk sexual activities.</td>
<td>131</td>
<td>69</td>
<td>1.65</td>
<td>82.5</td>
<td>M</td>
</tr>
<tr>
<td>2.8</td>
<td>- Lack of personal hygiene and neglect</td>
<td>160</td>
<td>40</td>
<td>1.8</td>
<td>90</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>Symptoms of cervical cancer:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Persistent pelvic pain</td>
<td>80</td>
<td>120</td>
<td>1.4</td>
<td>70.0</td>
<td>L</td>
</tr>
</tbody>
</table>
Cont... Table (1): Assessment of women’ knowledge related to the early detection

<table>
<thead>
<tr>
<th></th>
<th>Persistent vaginal secretions</th>
<th>99</th>
<th>101</th>
<th>1.49</th>
<th>74.75</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td>Pain during intercourse.</td>
<td>112</td>
<td>88</td>
<td>1.65</td>
<td>78.00</td>
<td>M</td>
</tr>
<tr>
<td>3.4</td>
<td>abnormal vaginal bleeding (bleeding after sex, bleeding after vaginal washing,</td>
<td>86</td>
<td>114</td>
<td>1.43</td>
<td>71.50</td>
<td>L</td>
</tr>
<tr>
<td>3.5</td>
<td>Rapid weight loss</td>
<td>66</td>
<td>134</td>
<td>1.33</td>
<td>66.50</td>
<td>L</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>1,579</td>
<td>1621</td>
<td>1.49</td>
<td>74.00</td>
<td>L</td>
</tr>
</tbody>
</table>

Table (1) demonstrate that there is Low knowledge of women, in total mean of scores (MS) which was (1.49) (74%) respectively.

Table (2): Women’s knowledge about methods, treatment and prevention of cervical cancer

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>N =200</th>
<th>Know</th>
<th>I do not know</th>
<th>MS</th>
<th>RS</th>
<th>Asses.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>- If cervical cancer is suspected, the doctor will ask the woman about the family’s medical history.</td>
<td>121</td>
<td>79</td>
<td>1.6</td>
<td>80.0</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>- do pap smear test (Papa Nicolao smear)</td>
<td>114</td>
<td>86</td>
<td>1.57</td>
<td>78.5</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>Every married woman should undergo cervical screening at least every two years,</td>
<td>120</td>
<td>80</td>
<td>1.6</td>
<td>80.0</td>
<td>M</td>
<td></td>
</tr>
</tbody>
</table>

2 | Treatment of cervical cancer:

| 2.1 | Electrical surgery | 91 | 109 | 1.45 | 72.7 | L |
| 2.2 | Laser surgery | 66 | 134 | 1.33 | 66.5 | L |
| 2.3 | Cryotherapy: Cold is used to eliminate abnormal cells. | 74 | 126 | 1.37 | 68.5 | L |
| 2.4 | Conical dislocation: The doctor removes a piece of cervical cone, to remove abnormal cells. | 52 | 148 | 1.26 | 63.0 | L |
| 2.5 | Hysterectomy | 120 | 80 | 1.6 | 80.0 | M |
| 2.6 | Curing overwhelming cancer: - Surgery | 72 | 128 | 1.36 | 68.0 | L |
| 2.7 | Radiation therapy | 71 | 129 | 1.35 | 67.75 | L |
| 2.8 | Chemotherapy | 72 | 128 | 1.36 | 68.0 | L |

3 | Prevention of cervical cancer:

| 3.1 | Obtain vaccination of HPV | 43 | 157 | 1.1 | 56.0 | L |
| 3.2 | - Pap smear examination | 128 | 72 | 1.64 | 82.0 | M |
| 3.3 | Chastity and non-sexual intercourse by both parties. | 130 | 70 | 1.65 | 82.5 | M |
| 3.4 | Quit Smoking. | 150 | 50 | 1.75 | 87.5 | M |
| **Total** | | 1424 | 1576 | 1.47 | 73.7 | L |
Table (2) demonstrate that there is Low knowledge to women, in the total mean of scores (MS) which was (1.47); (73.7%) respectively.

Table (3) Association between Level of Knowledge of Study Sample and Studied Variables

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Knowledge level</th>
<th>χ²</th>
<th>d. f</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unacceptable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acceptable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age / Years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>14</td>
<td>37</td>
<td>19.288</td>
<td>3</td>
<td>.000</td>
</tr>
<tr>
<td>30-39</td>
<td>30</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>34</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-above</td>
<td>20</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Read and write</td>
<td>8</td>
<td>0</td>
<td>35.774</td>
<td>4</td>
<td>.000</td>
</tr>
<tr>
<td>Primary</td>
<td>17</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>5</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Institute &amp; college</td>
<td>19</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>higher education</td>
<td>5</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupational status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teacher</td>
<td>28</td>
<td>42</td>
<td>17.298</td>
<td>2</td>
<td>.001</td>
</tr>
<tr>
<td>technician</td>
<td>61</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>employee</td>
<td>9</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Economic states</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>good</td>
<td>9</td>
<td>30</td>
<td>19.397</td>
<td>2</td>
<td>.000</td>
</tr>
<tr>
<td>moderate</td>
<td>72</td>
<td>68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>under moderate</td>
<td>17</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3) demonstration that there was statistically significant relationship among studied variable, and level of knowledge

**Discussion**

1 - Demographic characteristics of study sample.

Cervical cancer was the second most common cancer in women, cause high morbidity and mortality worldwide\(^{10}\)

In Iraq, significant knowledge gaps about the relative importance of cancer among the Iraqi community have been demonstrated suggesting a potential to take practical policy decisions that aim at promoting screening though elevating the level of awareness\(^{15}\) Throughout the present study, ) Regarding age group, more than half of women their ages were between (20-39) Years old. This result is agreeing with a study done in Kenya to assess women’s knowledge and attitudes related to cervical cancer the median age of sample was (66.3%) there aged18 to 39 years\(^{11}\)

Regarding to the level of education, the majority of them were Institute & college graduates. This result is similarly with a study it was done in Baghdad to assess teachers’ Knowledge regarding cervical cancer. \(^{13}\)

Furthermore, the study indicated that more than half of sample study in Moderate economics status.
This result agree with study done In Egypt shown that economic status(56%) had enough monthly income. (12)

According to Distribution of reproductive health history for women most of them (96.5%) was (12-14years) (Age at begin first menstrual cycle /years) this result disagrees with study done in Baghdad City who they found that the (2.5 %) had no pregnancy, 40%) had just one. (13) and ( (37.0%) of them had Family history of cancer the (14.5%) with Breast. Study done in Sudan states that can prevent cervical cancer, by prevent smoking, use oral contraceptive, and unsafe sex. (17)

2. Discussion of women’ knowledge related to the early detection.

There is Low of women knowledge, (table 1). this study also disagrees with study in Addis Ababa show that the odds of good knowledge about cervical cancer among government and nongovernmental organization employees were two times higher than among unemployed participants. (18) So, us study done in Chania assess that a total of 15.3% of the participants in this study indicated that they had never heard of cervical cancer previously. (19) so, us there is a deficit level of knowledge and awareness was documented concerning the epidemiology of cervical cancer (15)

3-Discussion the Women’s knowledge about methods, treatment and prevention of cervical cancer

There is low knowledge of women, regarding the total mean of scores (73.7%). In our study the results showed a deficiency in knowledge about methods, treatment, prevention cervical cancer some of the sample had less than (50%) from mean of scores the researcher opinion that the medical worker is one of the most important health knowledge provider and promoter. So, if the medical workers have unsatisfactory knowledge, inappropriate attitude and practice, they would not be spread the knowledge to the community , this study agree with study in Ethiopia, “ that women’s knowledge on cervical cancer was low, despite the high incidence of the disease in Ethiopia. Relatively, a large proportion of the study participants had favorable attitude towards cervical cancer screening attending primary, secondary school and college. (16)

4. Discussion the Association between Level of Knowledge of Study Sample and Studied Variables

There is a statistically significant relationship between studied variable, and level of knowledge this was not agree with study in Tanta, they found no statistically significant difference was found in relation to response to change, and a significant difference was found in relation to education and work. so, education and working environment increase all knowledge and awareness on cancer especially among married women as sexual health constitutes a taboo in our culture. and agreement to our findings, knowledge, Pap smear test were not associated with demographic variables as reported. (14) In addition, this result is corresponding with the study done in Baghdad, which revealed the educational status is positively associated with total knowledge of women on cervical cancer. who have primary, secondary and college/ university education were more likely to have best knowledge on cervical cancer than those who did not have. (15)

Conclusion

It was concluded is that that educated women of sample have deficit knowledge, towards cervical cancer and Pap smear.

Conflict of Interest: Nil

Source of Funding: the source of funding is self

Ethical Clearance: is obtained from the Technical Medicine Institute, Baghdad

Recommendation

1- increase coverage in cervical screening programs from Iraq ministry of health and directed towards women medical practitioners participants in the research - have been approved before the questionnaire is started

2- Long-term education programs should be made available to encourage the female population in the Iraq

3- Training should be supplied to nurses and primary care physicians to motivate screening.
References


Causative Microorganisms and Antibiotics Susceptibility in Neonatal Sepsis at Neonatal Intensive Care Unit: A Longitudinal Study from Diyala Governorate in Iraq

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Abstract

Background: Neonatal sepsis is classified into two types, early-onset and late-onset sepsis, depending on the time of appearance of the clinical features of neonatal sepsis.

Objective: We aim to detect the most common causative organisms of neonatal sepsis and to evaluate the corresponding antibiotics susceptibility in the Diyala governorate.

Patients and Methods: We prospectively collected a convenient sample of 106 sepsis-proven neonates from the neonatal intensive care unit at Al-Batool teaching hospital. We assessed all cases based on clinical features, laboratory investigations, and demographics.

Results: Late-onset neonatal sepsis was predominant (77.4%) among neonates, and it was significantly associated with neonatal prematurity and the mode of delivery at p-values of 0.03 and 0.045 respectively. Premature neonates and those who were the product of cesarean section were more prone to develop late-onset neonatal sepsis with a relative risk of 2.8 and 2.54 respectively. The most common causative microorganism of early-onset neonatal sepsis was found to be Escherichia coli in 45.8% of cases while those causing late-onset neonatal sepsis were mainly due to Gram-negative bacilli represented by Klebsiella pneumonia (46.3%) and Acinetobacter baumannii (24.4%). Multi-drug resistance was evident for most of the causative microorganisms.

Conclusion: To recapitulate, late-onset sepsis appeared was more common among Iraqi neonates, and it was significantly associated with the neonate prematurity and C-section mode of delivery.

Keywords: Anti-Bacterial Agents; Antibiotics Resistance; Causative Microorganisms; Intensive Care Units; Iraq; Middle East; Neonatal Sepsis; Neonatology.

Introduction

To date, neonatal sepsis is still a significant cause of neonatal mortality and morbidity, albeit effective modalities of diagnostics and therapeutics implemented by neonatologists worldwide, including those in the developed world. Neonatal sepsis is defined as the bacterial invasion of bloodstream causing non-specific systemic manifestations such as fever, respiratory problems, bradycardia or tachycardia, poor feeding, lethargy, irritability, seizures, abdominal distention, and unexplained jaundice. Neonatal sepsis is classified into two types, an early-onset and late-onset sepsis, depending on the time of appearance of the signs and symptoms of neonatal sepsis. Early-onset neonatal sepsis is diagnosed when the clinical manifestations of neonatal sepsis appear within the first 72 hours of life while the late-onset neonatal sepsis is diagnosed when they appear after the 72 hours of life. Early-onset neonatal sepsis is acquired before and during delivery, and it is either a trans-placental or an ascending infection within the birth canal. The microorganisms expected to cause early-onset sepsis are prevalent in the
maternal genital tract, including Escherichia coli (E. coli) and group B streptococci which are responsible for the majority of cases and may ascend to the amniotic fluid causing chorioamnionitis especially when there is a premature or a prolonged rupture of membranes (PPROM) (5). On the other hand, microbial agents inducing late-onset sepsis are either nosocomial or community-acquired infections and are transmitted to unfortunate neonates (1-5). Many factors predispose the neonates to get an infection (1). Risk factors for early-onset sepsis include maternal infections and febrile illnesses, chorioamnionitis, PPROM, prematurity and low birth weight (4). Risk factors for late-onset neonatal sepsis are invasive procedures, including surgical manipulation, intubation, central venous catheters, resuscitation, mechanical ventilation, and a prolonged stay in the neonatal care units (4).

The causative microorganisms variate spatially and temporally, and from a population to the other (1). Accurate identification of the source of infection, proper use of antimicrobial agents, and the study of antimicrobial susceptibility play essential roles in the prevention and control of neonatal sepsis and its complications, and this will increase the success rate of management of sepsis while reducing the economic burden on the holistic healthcare system, particularly in developing nations (6). It is imperative to know whether the newborn baby has got sepsis, or not, and to detect the type of causative invasive microorganisms, and to start the treatment as early as possible (7). A first interventional approach will reduce the risk of multidrug-resistant bacteria (MDR) which is defined as resistance to three or more antimicrobial classes (8). To date, there is no single test to diagnose neonatal sepsis with high specificity and sensitivity (1-3). Therefore, diagnostic tests must be in correlated in conjunction with assessing the risk factors and detecting the clinical signs of sepsis. Confirmatory laboratory tests include blood, urine, and cerebrospinal fluid cultures (CSF), as well as ESR, C-reactive protein (CRP), differential leukocyte profile, platelet count, latex agglutination tests and Polymerase Chain Reaction (PCR) (9).

In the present study, we aim to detect the causative microorganisms of neonatal sepsis and evaluate the antibiotic resistance in the neonatal intensive care unit in Al-Batool teaching hospital in the Diyala governorate. Our first hypothesis is that late-onset neonatal sepsis is more prevalent in our Neonatal intensive care unit (NICU) and mostly caused by Gram-negative bacilli. The second hypothesis is that multi-drug resistance exists among most of the causative microorganisms.

Materials and Methods

Using a convenient sampling procedure, we prospectively collected 106 sepsis-proven neonates in the neonatal intensive care unit of Al-Batool teaching hospital for the period from the 1st of December 2018 to the 1st of October 2019. We evaluated all the selected cases using clinical examination and laboratory investigations. The laboratory investigations included complete blood count (CBC), C-reactive protein (CRP), blood cultures, and chest x-ray (CXR). All cases enrolled in this study were diagnosed by the presence of at least three out of the following four criteria: 1) Presence of neonatal sepsis risk factors such as prematurity and chorioamnionitis. 2) Presence of two or more signs of the non-specific systemic manifestations such as fever, respiratory problems, bradycardia or tachycardia, poor feeding, lethargy, irritability, seizures, abdominal distention, and unexplained jaundice. 3) Positive CRP and abnormal CBC. 4) Positive culture (2). We also collected data concerning gestational age, the PPROM, mode of delivery, maternal fever, and sex of neonates. A professional laboratory technician collected blood samples from neonates with high clinical suspicion of sepsis for CRP, CBC, and blood culture. The technician collected approximately five cubic centimetres of fresh blood, using aseptic technique, from a peripheral vein from each neonate and sent for biochemical and microbiology studies for cultivation and subsequent processing. Besides, we collected samples of urine and CSF for culture procedures.

The blood samples were cultured under the aerobic condition at 37° C. The cultured blood was observed daily in the first three days to report any visible growth by detecting any one of the following: hemolysis, air bubbles, and coagulation of broth. At the same time, subcultures were made during three successive days on enriched and selective media including blood, chocolate, MacConkey, and mannitol salt agar plates and examined for microbial growth after 24 to 48 hours of incubation. The same procedures were repeated until
the 7th day before the blood culture was considered to be free of microorganisms. The obtained bacterial isolates were identified by microbiological methods including colony characteristics, Gram staining, and biochemical properties like catalase, coagulase and DNase production, hemolytic activity on blood agar plates, and growth on mannitol salt agar for Gram-positive bacteria. On the other hand growth on cetrimide agar, citrate utilization, urease, oxidase, and hydrogen sulfide production were used for Gram-negative bacteria. We used VITEK 2 AST-N327 kits (BIOMERIEUX/France) to validate the identification of Gram-negative bacteria. The results were read using API 32 GN reader. Antibiotics susceptibility was assessed by using the VITEK 2 AST-N327 identification kit in the VITEK 2 compact automated microbial detection system manufactured by bioMérieux France. The tested antibiotics with their minimal inhibitory concentration (MIC) were: Ampicillin 10\(\mu\)g, oxacillin 1\(\mu\)g, gentamicin 10\(\mu\)g, amikacin, vancomycin, ceftriaxone, cefotaxime, ceftazidime and cefoxitin with MIC of 30\(\mu\)g for each, ciprofloxacin 5\(\mu\)g, imipenem and meropenem with MIC of 10\(\mu\)g for each, azithromycin and erythromycin with MIC of 15\(\mu\)g for each, tetracycline (30\(\mu\)g), trimethoprim/sulfamethoxazole (1.25/23.75\(\mu\)g), fosfomycin (200\(\mu\)g), colistin (4\(\mu\)g), tigecycline (15\(\mu\)g) and piperacillin-tazobactam (100/10\(\mu\)g).

We analyzed the data collected by using IBM SPSS version 20, and we expressed the variables as frequencies and percentages. We implemented a Chi-squared test to study the relationship between the onset of neonatal sepsis and each of gestational age, PPROM, mode of delivery, maternal fever, and neonatal gender. We adopted a p-value of 0.05 as the cutoff margin for statistical significance in hypothesis testing.

**Results**

We collected a convenient sample of 106 neonates who had proven neonatal sepsis, and those patients were admitted to the neonatal care unit at Al-Batool teaching hospital from December 2018 to October 2019. We analyzed our data, using descriptive and inferential statistics, in connection with the onset of neonatal sepsis, possible risk factors, to examine the most common causative bacteria in the early and late-onset neonatal sepsis, and to discover the relative antimicrobial susceptibility. Male neonates were 52 (49.1%), and the female neonates were 54 (50.9%) (Table 1, Figure 1).

Among our sample, 24 (22.6%) were of early-onset sepsis, and the remaining 82 (77.4%) were of late-onset sepsis. Preterm neonates accounted for 76 cases (71.7%) and the term neonates attributed to 30 cases (28.3%). PPROM existed in 70 (66%) of cases while the remaining 36 (34%) had an intact membrane. A total number of 67 (63.2%) were a product of cesarean sections, and the remaining 39 (36.8%) were a product of vaginal delivery. There was a history of maternal fever in 69 (65.1%) of cases.

### Table 1: Descriptive parameters of the total sample.

<table>
<thead>
<tr>
<th>Data</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sepsis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early-onset</td>
<td>24</td>
<td>22.6%</td>
</tr>
<tr>
<td>Late-onset</td>
<td>82</td>
<td>77.4%</td>
</tr>
<tr>
<td><strong>Gestational age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm</td>
<td>76</td>
<td>71.7%</td>
</tr>
<tr>
<td>Term</td>
<td>30</td>
<td>28.3%</td>
</tr>
<tr>
<td><strong>State of membranes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruptured</td>
<td>70</td>
<td>66.0%</td>
</tr>
<tr>
<td>Intact</td>
<td>36</td>
<td>34.0%</td>
</tr>
<tr>
<td><strong>Mode of delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarian</td>
<td>67</td>
<td>63.2%</td>
</tr>
<tr>
<td>Normal</td>
<td>39</td>
<td>36.8%</td>
</tr>
<tr>
<td><strong>Maternal fever</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>69</td>
<td>65.1%</td>
</tr>
<tr>
<td>Absent</td>
<td>37</td>
<td>34.9%</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52</td>
<td>49.1%</td>
</tr>
<tr>
<td>Female</td>
<td>54</td>
<td>50.9%</td>
</tr>
</tbody>
</table>

In Al-Batool teaching hospital in Diyala governorate in Iraq, neonates in the neonatal intensive care unit were more prone to develop late-onset sepsis. The most common microorganisms causing late-onset neonatal sepsis included Klebsiella, Acinetobacter baumannii, coagulase-negative staphylococci (Staphylococcus epidermidis, Staphylococcus hominis,
and Staphylococcus haemolyticus), Staphylococcus aureus, and Enterobacter species which accounted for 46.34%, 24.4%, 17.1%, 3.7% and 3.7% of the late-onset neonatal sepsis respectively. Early-onset neonatal sepsis was mainly due to E. coli, Staphylococcus hominis, and Klebsiella, which represented 45.8%, 20.8%, and 12.5% of early-onset neonatal sepsis, respectively (Table 2).

**Table 2: The causative microorganisms in the early and late-onset neonatal sepsis.**

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Early-onset</th>
<th>Late-onset</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella</td>
<td>3 (7.3%)</td>
<td>38 (92.7%)</td>
<td>41</td>
<td>38.7%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>0 (0%)</td>
<td>20 (100%)</td>
<td>20</td>
<td>18.9%</td>
</tr>
<tr>
<td>E. coli</td>
<td>11 (100%)</td>
<td>0 (0%)</td>
<td>11</td>
<td>10.4%</td>
</tr>
<tr>
<td>Staphylococcus Epidermidis</td>
<td>1 (12.5%)</td>
<td>7 (87.5%)</td>
<td>8</td>
<td>7.5%</td>
</tr>
<tr>
<td>Staphylococcus hominis</td>
<td>5 (71.4%)</td>
<td>2 (28.6%)</td>
<td>7</td>
<td>6.6%</td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>5</td>
<td>4.7%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>4</td>
<td>3.8%</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>4</td>
<td>3.8%</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1</td>
<td>0.9%</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1</td>
<td>0.9%</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1</td>
<td>0.9%</td>
</tr>
<tr>
<td>Proteus species</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.9%</td>
</tr>
<tr>
<td>Pasturella pneumotropicus</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.9%</td>
</tr>
<tr>
<td>Coagulase-negative staph.</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1</td>
<td>0.9%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24 (22.6%)</strong></td>
<td><strong>82 (77.4%)</strong></td>
<td><strong>106</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

This study showed that the onset of the development of neonatal sepsis had a significant relationship with the gestational age and mode of delivery as p-values were equal to 0.03 and 0.045, respectively. Besides, the relative risk for preterm neonates to develop late-onset neonatal sepsis was 2.8 which means that there is a 180% increment in the probability of developing late-onset sepsis with prematurity compared with neonates who are born at full-term, while the relative risk for neonates who were products of cesarean sections to develop late-onset neonatal sepsis was 2.54. Thus they are more likely to develop late-onset neonatal sepsis by 154% more than neonates who were products of vaginal delivery (Table 3).
Table 3: The relation between the onset of neonatal sepsis and the possible risk factors.

<table>
<thead>
<tr>
<th>Data</th>
<th>Early-Onset</th>
<th>Late-Onset</th>
<th>Total</th>
<th>Chi-square value</th>
<th>P-value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm</td>
<td>13 (54.2%)</td>
<td>63 (76.8%)</td>
<td>76</td>
<td>4.699</td>
<td>0.03</td>
<td>2.8</td>
</tr>
<tr>
<td>Term</td>
<td>11 (45.8%)</td>
<td>19 (23.2%)</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24 (100%)</td>
<td>82 (100%)</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>State of membrane</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruptured</td>
<td>17 (70.8%)</td>
<td>53 (64.6%)</td>
<td>70</td>
<td>0.318</td>
<td>0.573</td>
<td>0.753</td>
</tr>
<tr>
<td>Intact</td>
<td>7 (29.2%)</td>
<td>29 (35.4%)</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24 (100%)</td>
<td>82 (100%)</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mode of delivery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarian</td>
<td>11 (45.8%)</td>
<td>56 (68.3%)</td>
<td>67</td>
<td>4.027</td>
<td>0.045</td>
<td>2.54</td>
</tr>
<tr>
<td>Normal</td>
<td>13 (54.2%)</td>
<td>26 (31.7%)</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24 (100%)</td>
<td>82 (100%)</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maternal fever</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>19 (79.2%)</td>
<td>50 (61%)</td>
<td>69</td>
<td>2.704</td>
<td>0.1</td>
<td>0.411</td>
</tr>
<tr>
<td>Absent</td>
<td>5 (20.8%)</td>
<td>32 (39%)</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24 (100%)</td>
<td>82 (100%)</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (54.2%)</td>
<td>39 (47.6%)</td>
<td>52</td>
<td>0.324</td>
<td>0.569</td>
<td>1.3</td>
</tr>
<tr>
<td>Female</td>
<td>11 (45.8%)</td>
<td>43 (52.4%)</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24 (100%)</td>
<td>82 (100%)</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The antibiotic resistances of the most common causative microorganisms, which were responsible for causing late-onset neonatal sepsis, were as following as shown in table 4: Klebsiella was resistant to penicillins, cephalosporins, and aminoglycosides, while it was sensitive to azithromycin, colistin, imipenem, and meropenem in 78%, 70.7%, 41.5% and 31.7% of cases respectively. Acinetobacter baumannii was 100% resistant to the tested penicillins, cephalosporins, and aminoglycosides, but it was sensitive to colistin and tigecycline in 95% and 60% of cases respectively. Staphylococcus epidermidis was resistant to the tested penicillins, cephalosporins, and aminoglycosides except for vancomycin since it appeared to be sensitive to vancomycin and tigecycline in 100% of cases.
respectively. Staphylococcus hemolyticus appeared to be resistant to most of the tested antibiotics, while it was sensitive to tigecycline, ciprofloxacin, and vancomycin in 80%, 60%, and 60% of cases respectively. Staphylococcus aureus was resistant to most of the tested antibiotics, but it was sensitive to ciprofloxacin, tigecycline, and vancomycin in 100%, 75%, and 75% of cases, respectively. Enterobacter species were resistant to most of the tested antibiotics, while it was sensitive to ciprofloxacin, tigecycline, and cefoxitin in 100%, 100%, and 50% of cases respectively.

Table 4: The antibiotic resistance of common organisms, based on blood culture, in late-onset sepsis.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Klebsiella</th>
<th>Acinetobacter baumannii</th>
<th>Staph. Epidermidis</th>
<th>Staph. hemolyticus</th>
<th>Staph. aureus</th>
<th>Enterobacter species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td></td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>40 (97.6%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>39 (95.1%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>38 (92.7%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>40 (97.6%)</td>
<td>20 (100%)</td>
<td>7 (87.5%)</td>
<td>5 (100%)</td>
<td>3 (75%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>33 (80.5%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>4 (80%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>0 (0%)</td>
<td>2 (40%)</td>
<td>1 (25%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>9 (22%)</td>
<td>14 (70%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>24 (58.5%)</td>
<td>18 (90%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>28 (68.3%)</td>
<td>20 (100%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>22 (53.7%)</td>
<td>19 (95%)</td>
<td>3 (37.5%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Methemprime</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>1 (12.5%)</td>
<td>1 (20%)</td>
<td>3 (75%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>33 (80.5%)</td>
<td>20 (100%)</td>
<td>5 (62.5%)</td>
<td>2 (40%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>41 (100%)</td>
<td>20 (100%)</td>
<td>7 (87.5%)</td>
<td>5 (100%)</td>
<td>3 (75%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>12 (29.3%)</td>
<td>1 (5%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>32 (78%)</td>
<td>8 (40 %)</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Tazocin</td>
<td>29 (70.7%)</td>
<td>17 (85%)</td>
<td>8 (100%)</td>
<td>5 (100%)</td>
<td>4 (100%)</td>
<td>4 (100%)</td>
</tr>
</tbody>
</table>
On the other hand, the antibiotic resistances of the most common causative microorganisms, which were responsible for causing early-onset neonatal sepsis, were as following as shown table 5: E. coli was resistant to the tested penicillins, cephalosporins and aminoglycosides except for amikacin since it appeared to be sensitive to meropenem, imipenem, amikacin and ciprofloxacin in 90.9%, 81.8%, 81.8% and 63.6% of cases respectively. Staphylococcus hominis was resistant to most of the tested antibiotics, but it showed 100% sensitivity to each of tigecycline and metheprome. Proteus species were 100% resistant to the tested antibiotics except for amikacin, meropenem, fosfomycin, and tazocin, where they were 100% sensitive for these antibiotics. Pasteurella pneumotropica was 100% resistant to the tested antibiotics except for amikacin and meropenem since it appeared to be 100% sensitive for each one of them.

Table 5: The antibiotic resistance of common organisms, based on blood culture, in early-onset sepsis.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli</th>
<th>Staph. hominis</th>
<th>Proteus species</th>
<th>Pasteurella pneumotropica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>9 (81.8%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>8 (72.7%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>9 (81.8%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2 (18.2%)</td>
<td>7 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>11 (100%)</td>
<td>6 (85.7%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>2 (18.2%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1 (9.1%)</td>
<td>7 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>9 (81.8%)</td>
<td>6 (85.7%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Metheprome</td>
<td>6 (54.5%)</td>
<td>5 (71.4%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4 (36.4%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>11 (100%)</td>
<td>7 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>9 (81.8%)</td>
<td>7 (100%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>11 (100%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Tazocin</td>
<td>6 (54.5%)</td>
<td>7 (100%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>
Discussion

Neonatal sepsis remains the major cause of morbidity and mortality in most of the neonatal intensive care units internationally. Hence, we should continually update our knowledge on the causative microorganisms, possible risk factors, and the emergence of multi-drug resistance microbial strains which drastically affect the outcome of management. Physicians should aim to facilitate the emergence of a potential empirical antibiotic therapy depending on the knowledge of their epidemiological factors and the antimicrobial susceptibility in connection with spatial and temporal epidemiology of neonatal sepsis (6). Our study showed that the Iraqi in the Diyala governate were more susceptible to develop late-onset neonatal sepsis (77.4%). Besides, the onset of the development of neonatal sepsis had a significant relationship with the gestational age and mode of delivery as p-values were equal to 0.03 and 0.045, respectively. Thus premature neonates were more prone to develop late-onset neonatal sepsis than term neonates as the relative risk was 2.8, and those who were the product of cesarean section appeared to be at a higher risk of developing late-onset neonatal sepsis as the relative risk was 2.54. Furthermore, multi-drug resistance was a problem for most of the causative microorganisms. The most common causative microorganism of early-onset neonatal sepsis was Escherichia coli in 45.8% of cases, and the second most common causative microorganism was Staphylococcus hominis which is a member of coagulase-negative staphylococcus and was present in 20.8% of cases. In contrast, those causing late-onset neonatal sepsis were principally Gram-negative bacilli, represented by Klebsiella pneumoniae (46.34%), Acinetobacter baumannii (24.4%), and coagulase-negative staphylococci (17.1%).

Jiang et al. (2014) mentioned that late-onset neonatal sepsis occurred in 71.9% of cases, and late-onset neonatal sepsis was more common in premature infants (11). In Kosovo, Julia (2018) found that the incidence rate of neonatal sepsis was 18.9% among them, 63.6% were EOS, and 34.6% were LOS (12). In Norway, Ronnestad (2005) reported that the incidence of LOS was 19.7% among neonates with low birth weight less than 1500gm and the most common pathogens were coagulase-negative staphylococci followed by Candida species (13). In Croatia, Stemberger and tešović (2012) found that preterm infants were more prone to both EOS and LOS and the most common causative agents of EOS were gram-negative rods including E. coli, Klebsiella species, and Pseudomonas while those causing LOS were staphylococci and enterococci (14). Similarly, Gowda et al. (2017) found that late-onset neonatal sepsis had a significant relationship with the prematurity (15). Kilani Basamad (2000) reported that E. coli was the most common causative agent of early-onset sepsis (16). In Australia, Bray et al. (2019) stated that E. coli and GBS were the most common causative agents of EOS in preterm and term babies, respectively (17). Berardi A. et al. (2019) mentioned that LOS was significantly associated with prematurity in Italian and the most common causative organisms were coagulase-negative staphylococci, E. coli, Staphylococcus aureus and Enterobacteriaceae (18). In India, Dudeja (2020) stated that in developing countries the more commonly encountered pathogens in causing neonatal sepsis were Gram-negative organisms represented by Acinetobacter and Klebsiella species, as well as E. coli (19). In 2011, Stoll and coworkers stated that the most significant pathogen in preterm infants was E. coli (20). Jiang and colleagues (2014) declared that the most encountered microorganism in the early onset neonatal sepsis were group B streptococci and E. coli (11). In 2015, Ansari and collaborators confirmed that the coagulase-negative staphylococcus, Staphylococcus aureus, Acinetobacter species, and Klebsiella pneumoniae were the most common etiological agents of late-onset neonatal sepsis (21). Jiang et al. (2014) stated that among the most common pathogens causing late-onset neonatal sepses were Klebsiella pneumoniae and Acinetobacter baumanii (11). On the other hand, Lee and associates (2004) stated that they did not encounter E. coli septicemia since early-onset neonatal sepsis was minimal in their study sample (22). Arild Ronnestad and Tore Gunnar Abraha (1998) found that coagulase-negative staphylococci were the main causative organism in the early and late-onset neonatal sepsis (23). Berger et al. (1998) reported that the most common pathogen causing early-onset sepsis was group B streptococcus (24).

These heterogeneities of the results from those existing studies might associate with the geographic and temporal variations of specific populations, and other differences related to demographics, ethnicity, the climate, and the environment, and the overall status of
the healthcare system. Our study has some limitations, including the relatively small sample size from a single tertiary healthcare centre using a covenant sampling method, the implementation of convenient sampling, and the lack of random selection of patients. Hence, future studies, including randomized controlled trials and meta-analytic studies in combination with predictive modelling and machine learning algorithms, are essential (25). Furthermore, the prolonged hospital stay, improper application of infection control programs, overcrowding of neonates, and the improper use of empirical therapy might be the aetiology behind the increment in the incidence rate of late-onset neonatal sepsis and the spread of hospital-acquired infections in our hospital. Therefore, regular and vigilant monitoring, evaluation of the neonatal intensive care unit measures, and reviewing the proper use of antimicrobial therapies are necessary to prevent and eliminate late-onset neonatal sepsis.

Conclusions

Late-onset neonatal sepsis appeared to be more common in our tertiary healthcare institute than early-onset neonatal sepsis. Klebsiella pneumonia, Acinetobacter baumannii, staphylococcus epidermidis, and Staphylococcus haemolyticus were the most common causative microorganisms. Late-onset neonatal sepsis appeared to be significantly associated with the prematurity and caesarian section. The increment in the incidence of late-onset neonatal sepsis and the antimicrobial resistance, including the horrific multi-drug resistance, necessitates the avoidance of overcrowding of neonates in intensive care units, minimizing the use of invasive interventional, the unnecessary use of broad-spectrum antibiotics, and the prolonged duration of treatment. Indeed, the application of infection control programs in the neonatal intensive care unit is critical to prevent and the development of neonatal sepsis.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: This study has no external funding.

Ethical Considerations

The ethics committee and the institutional review board of Al-Batool teaching hospital approved the study in 2018. The authors carried out the work described in this manuscript following the Code of Ethics of the World Medical Association (Declaration of Helsinki) on medical research involving human subjects, the EU Directive (210/63/EU), and the uniform requirements for manuscripts submitted to biomedical journals and the ethical principles defined in the Farmington Consensus of 1997. We acquired informed consent from the parents, or the guardians, of each patient. The patients were not subjected to further risk, and we dealt with their information dealt with complete confidentiality, and only the data curator had access to them, while other researchers were blinded.

References


A Study Evaluating Correlation between Umbilical Cord Attachment on Placenta in Normotensive and Hypertensive Pregnant Females and its Effects on Fetus

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Abstract

Introduction: Well nourished newborn is a reflection of adequate placental function. The umbilical cord that connects fetus and placenta can attach itself to placenta at different placenta. The incidence of central, eccentric, marginal and velamentous cord insertion is 18%, 73%, 7% and 1-2%, respectively. This insertion type significantly influences fetal growth and pregnancy outcomes.

Methodology: This study was conducted in Department of Anatomy of SBKS MIRC, Vadodara. This was a comparative study between normotensive and hypertensive group in which 500 subjects, in each group were included. Insertion of umbilical cord on placenta was determined and fetal growth and outcome with type of insertion were correlated.

Results: The two groups were comparable in terms of demographics. Higher proportion of those in hypertensive group had marginal insertion of umbilical cord (23.40%) as compared to 2.90% in normotensive group. Mean systolic (150.10±7.51mmHg) as well as diastolic blood pressure (91.23±4.00mmHg) was higher in those with marginal insertion of placenta. Mean fetal birth weight and APGAR score at birth and 5 minute and proportion of fetus reaching full term, live births were low in those in the hypertensive group especially in those with marginal insertion of placenta.

Conclusion: Thus it can be concluded that abnormal attachment of umbilical cord on placenta has significant impact on fetal growth and influences the outcomes of pregnancy directly and indirectly.

Key word:- Placenta, Umbilical cord, Cotyledon, Hypertension, Hypotension, Fetus.

Introduction

Adequate placental function results in well nourished newborn. Umbilical cord connects fetus and placenta and delivers oxygen and nutrients, throughout pregnancy, to the developing fetus. Thus, development of the umbilical cord determines and influences fetus growth. The site of cord insertion can be central, eccentric, marginal(Battledore) or velamentous (into fetal membranes) each occurring with an incidence 18%, 73%, 7% and 1-2%, respectively. [1] The attachment is considered marginal when cord attaches itself to placenta within 20mm from the placental edge. The insertion is called velamentous when umbilical cord inserts in to the chorio-amniotic membranes instead of placental mass. [2]
Two different theories can explain this variation in attachment: i) “placental migration theory or trophotropism”, which states that, to achieve better perfusion, placenta migrates towards the richly vascularised areas as the gestation advances. ii) “blastocyst polarity theory”, which specifies that malpositioning of blastocyst during implantation results in abnormal cord insertion. [3]

Various literatures have debated the significance of abnormal insertion of umbilical cord. It has been reported that marginal insertion is associated with increased frequency in abortions, malformed foetuses. It is also correlated with neonatal asphyxia and premature labour. Velamentous cord insertion results in lack of protection of Wharton’s jelly to the vessels and this makes the vessels prone to rupture and/or compression and can thereby cut off umbilical blood flow, acutely, which increases risk of perinatal death. [4]

Poor obstetric outcomes are observed in those with abnormal cord insertion. There have been reports of rise in fetal malformations, birth of neonates that are low weight, preterm induction of labor, intrauterine growth restriction, vasa previa, low APGAR scores and intrapartum complications. Shanklin DR et al, reported that in newborn weighing less than 2.5 kg, velamentous or marginal umbilical cord insertion was common. [5] Rath et al observed that hypertensive mothers’ commonly have marginal insertion of cord. [6]

We conducted this study to evaluate types of umbilical cord insertions on placenta in normotensive and hypertensive pregnant females and correlate this finding with fetal outcomes.

Methodology

This study was conducted in Department of Anatomy of SBKS MIRC, Vadodara. This was a comparative study between normotensive and hypertensive group in which 500 subjects, in each group, were included. It took five years for the study to get completed and the period of study was from Jan’12 to Dec’17. Fetal parameters were recorded. Morphology and Morphometric of placenta was evaluated. Insertion site of umbilical cord was noted. We calculated minimum distance between placental margin and insertion site of umbilical cord using a measuring scale and the same was labelled as ‘d’. The mean radius, denoted as ‘r’, was calculated from the surface. Insertion percentage was calculated using the formula: \((d/r) \times 100\). High insertion percentage suggests central insertion on the other hand low insertion percentage was suggestive of marginal insertion. The insertion was categorised as central (76-100%), eccentric-lateral (51-75%), eccentric-medial (26-50%) and marginal (0-25%). The collected data was entered into MS Excel and was analysed. Mean + SD and frequencies were calculated wherever appropriate. The level of significance was estimated using unpaired ‘t’ test.

Results

A total of 1000 cases, 500 hypertensives and 500 normotensive pregnant females were enrolled. In the hypertensive group, 25% (n=125), 50% (n=250) and 25% (n=125) had gestational hypertension, pre-eclampsia and eclampsia, respectively.

Patients enrolled were in 20 - 35 years age range.

It was seen that significantly higher proportion of patients in hypertensive group had marginal insertion of placenta as shown in Table 1.

<table>
<thead>
<tr>
<th>Site of Insertion of Placenta</th>
<th>Normotensive</th>
<th>Hypertensive group</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>GH</td>
<td>N</td>
</tr>
<tr>
<td>Central</td>
<td>215</td>
<td>21.50%</td>
<td>16</td>
<td>1.60%</td>
</tr>
<tr>
<td>EM</td>
<td>123</td>
<td>12.30%</td>
<td>21</td>
<td>2.10%</td>
</tr>
<tr>
<td>EL</td>
<td>133</td>
<td>13.30%</td>
<td>30</td>
<td>3.00%</td>
</tr>
<tr>
<td>Marginal</td>
<td>29</td>
<td>2.90%</td>
<td>58</td>
<td>5.80%</td>
</tr>
</tbody>
</table>
EM - Eccentric Medial; EL - Eccentric Lateral

The mean systolic blood pressure was higher in those in Marginal insertion of placenta as compared to other groups as shown in table 2.

**Table 2: Site of Insertion of Placenta and Mean blood pressure**

<table>
<thead>
<tr>
<th>Mean BP (mmHg)</th>
<th>Normotensive (Mean±SD)</th>
<th>Hypertensive group (Mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP</td>
<td>DBP</td>
<td>SBP</td>
</tr>
<tr>
<td>Central</td>
<td>123.09±4.11</td>
<td>82.23±4.07</td>
<td>148.13±5.72</td>
</tr>
<tr>
<td>EM</td>
<td>122.87±4.24</td>
<td>82.37±4.34</td>
<td>148.67±6.86</td>
</tr>
<tr>
<td>EL</td>
<td>123.29±4.10</td>
<td>82.51±3.82</td>
<td>152.43±6.71</td>
</tr>
<tr>
<td>Marginal</td>
<td>122.83±3.98</td>
<td>81.66±3.83</td>
<td>151.07±7.06</td>
</tr>
</tbody>
</table>

EM - Eccentric Medial; EL - Eccentric Lateral

The mean birth weight of the neonates was low in those born in marginal attachment of placenta as shown in table 3.

**Table 3: Site of Insertion and Mean fetal body weight**

<table>
<thead>
<tr>
<th>Mean Birth Weight (Kg) (Mean±SD)</th>
<th>Normotensive</th>
<th>Hypertensive group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GH</td>
<td>PE</td>
<td>Eclampsia</td>
</tr>
<tr>
<td>Central</td>
<td>2.90±0.53</td>
<td>2.21±1.08</td>
<td>2.47±0.55</td>
</tr>
<tr>
<td>EM</td>
<td>2.87±1.03</td>
<td>2.17±0.90</td>
<td>2.41±0.35</td>
</tr>
<tr>
<td>EL</td>
<td>2.81±1.00</td>
<td>2.06±0.90</td>
<td>2.35±0.55</td>
</tr>
<tr>
<td>Marginal</td>
<td>2.43±0.68</td>
<td>2.04±0.98</td>
<td>2.31±0.41</td>
</tr>
</tbody>
</table>

EM - Eccentric Medial; EL - Eccentric Lateral

In both the groups, a significantly higher proportion of neonates in those with marginal attachment had weight less than 2.5kg as shown in table 4.
Table 4: Correlation between insertion of placenta and neonate weight

<table>
<thead>
<tr>
<th>Neonate weight</th>
<th>Normotensive</th>
<th>Hypertensive group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2.5 kg</td>
<td>&gt;=2.5kg</td>
</tr>
<tr>
<td>Central</td>
<td>6</td>
<td>209 1.20%</td>
</tr>
<tr>
<td>Eccentric Medial</td>
<td>1</td>
<td>122 0.20%</td>
</tr>
<tr>
<td>Eccentric Lateral</td>
<td>3</td>
<td>130 0.60%</td>
</tr>
<tr>
<td>Marginal</td>
<td>17</td>
<td>12 3.40%</td>
</tr>
</tbody>
</table>

The APGAR score at birth and at 5 minutes was low in those with marginal attachment of placenta as compared to those with central attachment of placenta as shown in table 5.

Table 5: Site of insertion and APGAR score at birth

<table>
<thead>
<tr>
<th>APGAR score at birth(Mean±SD)</th>
<th>Normotensive Group</th>
<th>Hypertensive group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GH</td>
<td>PE</td>
</tr>
<tr>
<td>Central</td>
<td>8.1±1.03</td>
<td>6.26±2.4</td>
</tr>
<tr>
<td>Eccentric Medial</td>
<td>7.88±1.4</td>
<td>6±2.35</td>
</tr>
<tr>
<td>Eccentric Lateral</td>
<td>7.59±1.19</td>
<td>5.9±2.84</td>
</tr>
<tr>
<td>Marginal</td>
<td>7.55±1.49</td>
<td>5.53±2.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APGAR score at 5 minutes (Mean±SD)</th>
<th>Normotensive Group</th>
<th>Hypertensive group</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>PE</td>
<td>Eclampsia</td>
</tr>
<tr>
<td>Central</td>
<td>9.55±0.72</td>
<td>8.24±3.22</td>
</tr>
<tr>
<td>Eccentric Medial</td>
<td>9.36±1.41</td>
<td>7.69±3.14</td>
</tr>
<tr>
<td>Eccentric Lateral</td>
<td>9.22±1.16</td>
<td>7.52±3.79</td>
</tr>
<tr>
<td>Marginal</td>
<td>9.12±1.53</td>
<td>7.2±3.33</td>
</tr>
</tbody>
</table>

Preterm deliveries, IUDs and NICU admissions were common in those with marginal attachment of placenta as shown in table 6 in both the groups.
Table 6: Correlation of fetal outcomes with site of attachment of placenta

<table>
<thead>
<tr>
<th>Term at birth</th>
<th>Normotensive</th>
<th></th>
<th>Hypertensive group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full term</td>
<td>Pre-term</td>
<td>Full term</td>
<td>Pre-term</td>
</tr>
<tr>
<td>Central</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>18.20%</td>
<td>33</td>
<td>3.30%</td>
</tr>
<tr>
<td>Eccentric Medial</td>
<td>113</td>
<td>11.30%</td>
<td>10</td>
<td>1.00%</td>
</tr>
<tr>
<td>Eccentric Lateral</td>
<td>117</td>
<td>11.70%</td>
<td>16</td>
<td>1.60%</td>
</tr>
<tr>
<td>Marginal</td>
<td>24</td>
<td>2.40%</td>
<td>5</td>
<td>0.50%</td>
</tr>
<tr>
<td>Birth status</td>
<td>Live Birth</td>
<td></td>
<td>IUD</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>211</td>
<td>21.10%</td>
<td>4</td>
<td>0.40%</td>
</tr>
<tr>
<td>Eccentric Medial</td>
<td>122</td>
<td>12.20%</td>
<td>1</td>
<td>0.10%</td>
</tr>
<tr>
<td>Eccentric Lateral</td>
<td>131</td>
<td>13.10%</td>
<td>2</td>
<td>0.20%</td>
</tr>
<tr>
<td>Marginal</td>
<td>29</td>
<td>2.90%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>ICU admission</td>
<td>Required</td>
<td></td>
<td>Not-required</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.50%</td>
<td>206</td>
<td>20.60%</td>
</tr>
<tr>
<td>Eccentric Medial</td>
<td>4</td>
<td>0.40%</td>
<td>118</td>
<td>11.80%</td>
</tr>
<tr>
<td>Eccentric Lateral</td>
<td>4</td>
<td>0.40%</td>
<td>127</td>
<td>12.70%</td>
</tr>
<tr>
<td>Marginal</td>
<td>1</td>
<td>0.10%</td>
<td>28</td>
<td>2.80%</td>
</tr>
</tbody>
</table>

Discussion

Various studies have implicated role of marginal insertion of umbilical cord in the placenta with induction of hypertension.\[7, 8\] In the present study it was seen that marginal attachment of placenta was common in those in hypertensive group. However, ‘eccentric’ insertion of the umbilical cord was reported in both normotensive and hypertensive groups by certain authors.\[3, 9\]. The difference may be due to the fact that these authors enrolled pre-eclamptic females in hypertensive group where our study enrolled those with gestational hypertension and eclampsia as well. And as confirmed in the findings of Udainia A. et al with the increase in the severity of PIH, the umbilical cord insertion on placenta shifts marginally and may even become velamentous.\[9\]

Additionally, we observed that in those with marginal insertion of placenta the blood pressure was on higher side as compared to other type of insertions in both the groups. There are two different theories, one that
suggest that hypertension lead to marginal attachment and other that marginal attachment of placenta induces hypertension. Cai LY et al and Jain A et al have reported that hypertension is induced by abnormal insertion of placenta. [3, 9]

Authors like Udaina A et al, Jain A et al have reported low birth weight in those with marginal attachment [3, 9]. In the present study also it was observed that fetal weight was low in those with hypertensive group as compared to normotensive group and was lowest in those with marginal attachment of placenta. Thus our finding is consistent with literature that abnormal cord insertion is correlated with intrauterine growth restriction (IUGR), this may be because abnormal insertion of umbilical cord may impact the nutrient and oxygen transfer across placenta. [3, 10] The reason for abnormal nutrient transfer may be that the in such circumstances of abnormal attachment, density of vessels in placenta is low as against when the insertion is normal; also an increased vascular resistance may be encountered on account of long fetal stem vessels. [3]

We observed low mean APGAR scores at birth and at 5 minutes in those in the hypertensive group, especially in those with marginal attachment of placenta. Similar to our study Heinonen S et al observed low APGAR score at 1 minute and 5 minutes after birth in those with abnormal placental attachment as compared to those with normal attachment of placenta. [12]

Brody S, et al., observed that battledore placenta may sometimes be responsible for the premature initiation of labour. This may be because of interference with fetal circulation which causes fetal embarrassment and upset the balance of opposing forces existing between the uterus, placenta, and fetus for the maintenance of pregnancy and thus induces labor prematurely. [11] In the present study, it was observed that preterm deliveries occurred commonly in the hypertensive group and that more frequently in those with marginal insertion of placenta. In the study by Heinonen S et al, prematurity was observed in 13.9% of the cases with abnormal placental attachment as compared to 6.1% of the cases in those with normal placental attachment. [12] Similar to our study the authors, Heinonen S et al, also observed higher fetal mortality and increased ICU requirement in those with abnormal placental attachment.

Conclusion

Thus it can be concluded that abnormal attachment of umbilical cord on placenta has significant impact on fetal growth and influences the outcomes of pregnancy directly and indirectly.

Ethical Clearance- Taken from Sumandeepl Vidyaapeeth University committee

Source of Funding- Self

Conflict of Interest - Nil

References


A Cross Sectional Descriptive Study of Analysis of Lambdoid And Squamous Sutures Closure by Ct Scan for Age Estimation

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1Associate Professor, 2Professor & Head, Dept of Forensic Medicine & Toxicology, 3Assistant Professor, Department of Radiology, SBKSMIRC, Sumandeep Vidyapeeth, Vadodara

Abstract

Background—Identification is fixation of an individuality by various physical and biological parameters. An individual’s age determination is very important in various civil and criminal medicolegal cases. Age estimation by cranial sutures closure is an old method. This study was conducted to analyze closure of lambdoid and squamous sutures for age estimation in living individuals by CT scan.

Objectives—To study the pattern of closure of lambdoid and squamous sutures with relation to age of person. To detect bilateral and bisexual variations in same.

Materials & Methods—We had done a descriptive cross-sectional study by CT scan head of 130 living adult persons and analyzed them for closure status of lambdoid and squamous sutures in co-relation to age.

Results—In Lambdoid suture complete closure observed 5yrs earlier in upper 2/3rd(L1&L2) parts of right side than left side in both sex while while complete closure observed very late ages(>75yrs) or not at all(lapsed union) in lower 1/3rd(L3) part. Both sides of Squamous sutures follow more or less same pattern for closure in relation to age and sex while complete closure observed at very late ages(>75yrs) or not at all(lapsed union).

Conclusion—Closure of Lambdoid and Squamous sutures should be used only as corroborative to other criteria and evidences for age estimation as they might be unreliable as standalone until proven otherwise.

Key Words: Age determination, Lambdoid suture, Squamous suture, CT scan, Identification.

Introduction

Age is a primordial parameter for identification of an individual. Determination of exact age is needed for living individuals in various civil disputes like employment, marriage, attainment of majority, property dispersal, voting rights, senior citizenship, competency as witness and in various crime disputes like rape, infanticide, kidnapping, juvenile delinquency, mercy for capital punishment and criminal responsibility1. At different periods of life, different methods are utilized for scientific age estimation like weight, head-circumference and crown heel length in fetus and newborn; dentition and radiological examination of bones in children and adults under age of 25 years. Scientific age estimation is more complex after age of 25 years. Only few parameters are helpful in this stage of life such as closure of cranial sutures, changes of symphysis pubis, appearance and fusion of segments of sternum and sacrum2-5.

Closure of the skull suture is considered a fairly reliable index for age determination between 25 and 40 years of age. It can estimate age only in range of a decade2,3.

Morphological methods are studied & being used in autopsy cases. Radiological methods like X-ray, computed tomography(CT) scans, MRI and 3D constructed CT scanning & MRI, though costly is being used now in living persons and in virtual autopsy.

Materials and Methods

This study was conducted during September 2012 to October 2014 by Forensic Medicine & Toxicology
department in collaboration of Radiology department at Govt. Medical College & New Civil Hospital, Surat. Institutional ethics committee approval obtained for this research. We included the living adult subjects of of 18 yrs & above with minimum two valid age or birthdate proof documents e.g. birth certicate, driving licence, rationcard, aadharcard etc. Persons with medical history of head injury or disease with cranium damage. pregnant females were also excluded due to radiation hazards.

The age of subjects were ranging from 15 years to 92 years. For equal distribution of samples, we made total 13 strata of age groups with difference of 5 years of age i.e. 15-19yrs, 20-24yrs, ..., 70-74yrs and 75yrs and more. In each group, 5 male and 5 female subjects were enrolled by simple randomization. So, total 65 male and 65 female subjects were studied after obtaining informed consent. Because of high cost of CT scan imaging procedure and only healthy (cranium wise) subjects included in our study, the size of study sample remained quiet restricted. Axial CT scans of skull done at different level for bony window. CT scan machine used was 6/16 slice Siemen Company model ‘SOMATOM Emotion 6’. CT scan images were analysed for ectocranial fusion of segments of Lambdoid and Squamous sutures bilaterally.

The Squamous sutures were analysed as a whole on both sides whereas, based on the methods of Broca\(^6\) and Singer\(^7\) the Lambdoid suture was subdivided into three parts upper 1/3rd-L1-Pars Lambdica, middle 1/3rd-L2-Pars Intermedia & lower 1/3rd-L3-Pars Asterica on both sides.

**Observations & Results**

Table 1: Age and gender co-related findings for ectocranial closure status in Lambdoid suture.
The LR1 part of suture noted open in subjects of age group of 35-39 years and younger in both sex, but it was in closing process in early fifties and found closed in majority (4 or more in strata) in age group of 60-64 years and older in both sex. The closure starting observed earliest at 36 years of age in male and 35 years of age in female and complete closure observed earliest at 45 years in male and 51 years in female. The closure starting observed earliest at 36 years of age in male and 35 years of age in female and complete closure observed earliest at 45 years in male and 51 years in female. The LR2 part of suture was open in subjects of age group of 40-44 years and younger in both sex, but it was in closing process in late forties and found closed in majority in age group of 65-69 years and older in both sex. The closure starting observed earliest at 36 years of age in male and 35 years of age in female and complete closure observed earliest at 45 years in male and 51 years in female. The LR3 part of suture was open in subjects of age group of 40-44 years and younger in both sex, but it was in closing process in late fifties and but no complete closure found in clear majority till last age group of study in both sex. The closure starting observed earliest at 45 years of age in in both sex and complete closure observed earliest at 60 years in male and 65 years in female.

The LL1 part of suture was open in subjects of age group of 30-34 years and younger in both sex, but it was in closing process in late forties and found closed in majority (4 or more in strata) in age group of 65-69 years and older in both sex. The closure starting observed earliest at 36 years of age in male and 35 years of age in female and complete closure observed earliest at 45 years in male and 51 years in female. The LL2 part of suture was open in subjects of age group of 40-44 years and younger in both sex, but it was in closing process in late fifties and found closed in majority in of 65-69 years and older in both sex. The closure starting observed earliest at 40 years of age in in male and 44 years in female while complete closure observed earliest at 48 years in male and 51 years in female. The LL3 part of suture was open in subjects of age group of 40-44 years and younger in both sex, but it was in closing process in late fifties and no complete closure found in clear majority till last age group of study in both sex. The closure starting observed earliest at 45 years of age in in both sex and complete closure observed earliest at 60 years in male and 65 years in female. Lambdoid suture closure observed from Lambda point towards base of skull on both sides.
Table 2: Age and gender co-related findings for ectocranial closure status in Squamous suture.

<table>
<thead>
<tr>
<th>Age Group (In Years)</th>
<th>Sex</th>
<th>Squamous (Temporo-Parietal) Suture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SQR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Open (n)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>15-19</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
</tr>
<tr>
<td>20-24</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
</tr>
<tr>
<td>25-29</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
</tr>
<tr>
<td>30-34</td>
<td>M</td>
<td>5</td>
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<tr>
<td></td>
<td>F</td>
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</tr>
<tr>
<td>35-39</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
</tr>
<tr>
<td>40-44</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
</tr>
<tr>
<td>45-49</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
</tr>
<tr>
<td>50-54</td>
<td>M</td>
<td>5</td>
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<td>55-59</td>
<td>M</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3</td>
</tr>
<tr>
<td>60-64</td>
<td>M</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2</td>
</tr>
<tr>
<td>65-69</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
</tr>
<tr>
<td>70-74</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>&gt;75</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>88</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SQL</th>
<th>Open (n)</th>
<th>In closing process (n)</th>
<th>Complete closure (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>
Table-2 describes the age and gender co-related findings for ectocranial closure status in Squamous or Temporo-Parietal suture.

The SQR(right squamous suture) was observed open till age group of 50-54 years and in closing process in late sixties but no complete closure found in clear majority till last age group of study in both sex. The closure starting observed earliest at 55 years in male and 54 years in female. Complete closure was observed earliest at 72 years in male and 70 years in female. The SQL(left squamous suture) was observed open till age group of 50-54 years and in closing process in late sixties but no complete closure found in clear majority till last age group of study in both sex. The closure starting observed earliest at 55 years in male and 54 years in female. Complete closure was observed earliest at 72 years in male and 65 years in female.

Table 3: Suture closure status in relation to age and age groups:

<table>
<thead>
<tr>
<th>Suture</th>
<th>Part Of Suture</th>
<th>Sex</th>
<th>Closure started earliest at age (years)</th>
<th>Suture completely closed observed earliest at age (years)</th>
<th>Suture completely closed observed in 4 or more out of 5 subjects at age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQR</td>
<td></td>
<td>M</td>
<td>55</td>
<td>72</td>
<td>&gt;75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>54</td>
<td>70</td>
<td>&gt;75</td>
</tr>
<tr>
<td>Squamous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SQL</td>
<td></td>
<td>M</td>
<td>55</td>
<td>72</td>
<td>&gt;75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>54</td>
<td>65</td>
<td>&gt;75</td>
</tr>
<tr>
<td>Lambdaid Suture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR1</td>
<td></td>
<td>M</td>
<td>36</td>
<td>45</td>
<td>60-64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>35</td>
<td>51</td>
<td>60-64</td>
</tr>
<tr>
<td>LR2</td>
<td></td>
<td>M</td>
<td>45</td>
<td>48</td>
<td>60-64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>45</td>
<td>50</td>
<td>65-69</td>
</tr>
<tr>
<td>LR3</td>
<td></td>
<td>M</td>
<td>45</td>
<td>60</td>
<td>&gt;75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>45</td>
<td>65</td>
<td>&gt;75</td>
</tr>
<tr>
<td>LL1</td>
<td></td>
<td>M</td>
<td>36</td>
<td>45</td>
<td>65-69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>35</td>
<td>51</td>
<td>65-69</td>
</tr>
<tr>
<td>LL2</td>
<td></td>
<td>M</td>
<td>44</td>
<td>48</td>
<td>65-69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>40</td>
<td>51</td>
<td>65-69</td>
</tr>
<tr>
<td>LL3</td>
<td></td>
<td>M</td>
<td>45</td>
<td>60</td>
<td>&gt;75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>45</td>
<td>65</td>
<td>&gt;75</td>
</tr>
</tbody>
</table>
Table-3 concise the findings described in earlier tables. In Lambdoid suture it was observed that closure complete 5yrs earlier in upper 2/3rd(L1&L2) parts of right side than left side in both sex while while complete closure observed very late ages(>75yrs) or not at all(lapsed union) in lower 1/3rd(L3) part. Both sides of Squamous sutures follow more or less same pattern for closure in relation to age and sex while complete closure seems occur at very late ages(>75yrs) or not at all(lapsed union).

**Discussion**

Here is the comparision of this study to other studies done by various researchers from different regions of India and other countries by various methods.

**Table 4: Comparision of various studies- Age of closure of Lambdoid suture**

<table>
<thead>
<tr>
<th>Researcher/Author</th>
<th>Year</th>
<th>Race</th>
<th>Age of closure(Yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vij K2</td>
<td>2011</td>
<td>Indian</td>
<td>55</td>
</tr>
<tr>
<td>Dikshit PC3</td>
<td>2007</td>
<td>Indian</td>
<td>50-60</td>
</tr>
<tr>
<td>Reddy KSN4</td>
<td>2007</td>
<td>Indian</td>
<td>50-60 in L1,L2 60-70 in L3</td>
</tr>
<tr>
<td>Nandy A5</td>
<td>2001</td>
<td>Indian</td>
<td>55</td>
</tr>
<tr>
<td>Mukharjee JB8</td>
<td>2011</td>
<td>Indian</td>
<td>55</td>
</tr>
<tr>
<td>Aggrawal A9</td>
<td>2014</td>
<td>Indian</td>
<td>45</td>
</tr>
<tr>
<td>Modi K10</td>
<td>2012</td>
<td>Indian</td>
<td>&gt;70(lapsed union)</td>
</tr>
<tr>
<td>Pillay VV11</td>
<td>2008</td>
<td>Indian</td>
<td>40-50</td>
</tr>
<tr>
<td>Krogman WM, Iscan MY12</td>
<td>1986</td>
<td>USA</td>
<td>60-69</td>
</tr>
<tr>
<td>Parmar P, Rathod G13</td>
<td>2012</td>
<td>Indian</td>
<td>45 to 55</td>
</tr>
<tr>
<td>Todd TW &amp; Lyon DW14</td>
<td>1924-25</td>
<td>USA</td>
<td>Start – 26 Complete – 31</td>
</tr>
<tr>
<td>Shetty U15</td>
<td>2008</td>
<td>Indian</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Present study</td>
<td>2014</td>
<td>Gujarat-Indian</td>
<td>60-69 L1,L2 &gt;75 L3</td>
</tr>
</tbody>
</table>

Table-4 shows comparision of various studies from India and USA regarding complete closure of Lambdoid sutures.
Table 5: Comparison of various studies- Age of closure of Squamous suture

<table>
<thead>
<tr>
<th>Researcher/Author</th>
<th>Year</th>
<th>Race</th>
<th>Age of closure(Yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dikshit PC3</td>
<td>2007</td>
<td>Indian</td>
<td>60 to 90 in both sex</td>
</tr>
<tr>
<td>Reddy KSN4</td>
<td>2007</td>
<td>Indian</td>
<td>80yrs in both sex</td>
</tr>
<tr>
<td>Nandy A5</td>
<td>2001</td>
<td>Indian</td>
<td>70yrs or above</td>
</tr>
<tr>
<td>Mukharjee JB6</td>
<td>2011</td>
<td>Indian</td>
<td>70yrs or above</td>
</tr>
<tr>
<td>Pillay VV9</td>
<td>2008</td>
<td>Indian</td>
<td>Around 60yrs</td>
</tr>
<tr>
<td>Parikh CK16</td>
<td>1990</td>
<td>Indian</td>
<td>60</td>
</tr>
<tr>
<td>Singh P et al17</td>
<td>2001-04</td>
<td>Panjab</td>
<td>55 to 65</td>
</tr>
<tr>
<td>Parmar P, Rathod G11</td>
<td>2012</td>
<td>Indian</td>
<td>60 to 70</td>
</tr>
<tr>
<td>Verma RK, Goyal MK, Kochar S18</td>
<td>2002</td>
<td>Rajasthan-Indian</td>
<td>80yrs</td>
</tr>
<tr>
<td>Moondra AK19</td>
<td>2000</td>
<td>Indian</td>
<td>&gt;75yrs</td>
</tr>
<tr>
<td>Present study</td>
<td>2013-14</td>
<td>Gujarat-Indian</td>
<td>&gt;75(lapsed union)</td>
</tr>
</tbody>
</table>

Table-5 shows comparison of various studies from India and USA regarding complete closure of Squamous sutures ectocranially at ages.

So, we can see that Lambdoid sutures and Squamous sutures closure noted at different ages in different populations.

**Limitations & Recommendation:**

Sample size is quiet small due to feasibility issues. More studies may be done in other regions enrolling identical populations to limit bias factors like climate, diet & nutrition, inheritance, race which was not considered in present study.

**Conclusion**

Cranial suture closure is fairly reliable method for age estimation in elderly age with Lambdoid suture & Squamous sutures. Very much variation is observed in terms of age of complete closure especially in lower 1/3rd of Lambdoid suture and Squamous suture. Incomplete closure and lapsed union seems common until at and over the age of 75yrs in same. No significant difference observed in age of closure of these sutures between male and female sex. However, upper two thirds of Lambdoid suture closure is observed 5yrs earlier on right side than left side. Closure of Lambdoid & Squamous sutures should be used only as corroborative to other criteria & evidences for age estimation.

**Conflict of Interest:** None declared.

**Source of Funding:** Self funded.

**References**

4. Reddy KSN. Essentials of forensic medicine and
toxicology. 32th ed. Hyderabad: K Sugunadevi; 2013, Identification; P.75-78.


Effectiveness of Laughter Therapy on Reduction of Stress among Nursing Students

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Abstract

Context: Stability of hormones are being effected by consistent stress in the human body which leads to changes in thoughts and situations that make the individual perplexed, restless or anxious. Laughter therapy is the antidote for stress. It helps to release serotonin in brain which is essential for the uplift of mood.

Aim: The aim of this research is to find out the efficacy of laughter therapy on the decline of stress.

Setting and design: Data collection for the commenced study was conducted at Sri Sukhmani College of Nursing and Amar Professional College of Nursing, Dyalpura, District Mohali, Punjab. A quantitative approach with “Quasi-experimental design” was adopted to conduct this research.

Methods and Material: Technique used for selecting the subjects was purposive sampling technique. 60 subjects were selected and sub grouped into experimental and control group (30 each). 5 point likert scale i.e. Sheldon Cohen”s (1983) Perceived Stress Scale which includes 10 items, was selected to evaluate the level of stress among nursing students.

Statistical analysis used: Descriptive and inferential statistics were used.

Results:

• Before implementation of laughter therapy, it was identified that stress scores were approximately same in both the groups.

• After implementation of therapy, it was identified that stress scores in the experimental group was lower than the control group.

Conclusions: Study concluded with the result that stress level is alleviating among student nurses with help of laughter therapy.

Key Words: Effect, Laughter therapy, Stress, Nursing students.

Introduction

Stress can be defined in a number of ways and every individual faces it in their everyday life. But the most important part of an individual is to manage it. Stress may lead to benefits or drawbacks; it leans on one’s view that how to perceive and take over it. If the stress is not managed adequately, sentiments of dejection, anxiety, and restlessness may occur. Stress can only be minimized by using adequate coping techniques which brings balance in the human body and mind.

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In the nutshell, Student nurses most commonly face the following stresses i.e.

- Societal stress,
- Monetary stress,
- College stress, and
- Clinical field stress.

Societal pressure doesn’t mean having individuals around you. It incorporates dread of speech, showdowns, and overseeing authority obligations.

Monetary stress is faced when students are in lack of resources (Example: Money Crisis).

Academic/college stress in students can be due to learning for finals concerning rank competition or dread of failure in finals. Clinical area stress includes clinical placements, fear of making mistakes, and interactions with other staff members.

Students confront numerous stressors and hindrances during student life. Nursing student encounter’s even “more stress” as compared to their buddies registered in other courses. Accordingly, various researchers proclaimed that level of stress is high in student nurses. Nursing students are prone to different types of stresses because of the ever-changing environment of college. Increased stress level is supposed to influence students’ wellbeing and scholastic functions.

So as to lessen the degree of stress, various relaxation techniques and exercises have been utilized. Among this laughter is considered as the finest stress busting. Laughter is the human’s best gift for coping and endurance. The silent strength of laughter is triggered whenever we laugh and need of laughter is much more in this stressful world. “Freud stated in his theory that laughter therapy releases tension and psychic energy”, it is a coping mechanism for when one is upset, angry or sad. 15 minutes of laugh is equaled to the benefit of two-hour sleep, 15 minutes laugh adds two days life span. It stimulates the brain, respiratory, nervous, hormonal, and muscular systems. Many researches evidenced that laugh increase the secretion of serotonin in brain which is essential for the uplift of mood. It also triggers the discharge of endorphins (body’s natural analgesic) and produces a general feeling of prosperity. Dr. Leeberk investigated that stress hormones can be decreased by therapy of laughter. According to Jhonson Thomas (2011) in Maharashtra, 70% of subjects have become short-tempered and suicide rates are inclined with respect to raise in age only due to stress, but there has recently been an alarming increase in self-destructive behaviors among youngsters because of stress.

Laughter therapy has various benefits in stress management like it helps to decrease the stress hormones like “cortisol, epinephrine (adrenaline), dopamine” and also reinforces the health-promoting hormones like “endorphins”. Laughter helps to discharge tensions, physically as well as emotionally; which also keeps the heart healthy. Laughter changes the emphasis beyond outrage, stress, and pessimistic feelings in an advantageous manner than any insignificant distractions. Laughter assists us to connect with others in easy means that can uplift the mood of people around us which may lead to healthy social interaction and a decline in the level of stress.

I. Based on own personal experience, the researcher felt that nursing students experience a lot of stress due to competition, topographical versatility, new way of life, exams, tests, grades, extended periods of time of contemplating, work, family and other individual responsibilities, students also face the difficulties of clinical practice and strict disciplinary lodging life, monetary burden, dispute with friends or classmates, scholastic pressure as well as in the clinical area as they have direct contact with patients. There are many physiological and psychological changes in the body due to stress that may lead to various mental and physical illnesses. As many of studies have revealed that there is an increasing number of suicide due to stress among students and many of the previous researches have shown the benefits of laughter therapy in reduction of stress, but few studies have shown its impact on nursing students, therefore need for conducting the study is recognized among nursing students.

Subjects and Methods

A quantitative research approach with a quasi-experimental research design was adopted to accomplish the objectives of the survey i.e. to determine the effectiveness in reduction of stress level among nursing students with assistance of laughter therapy. Control
group and manipulation (i.e. intervention) was included. But randomization was not done due to the non-availability of a large number of subjects with stress level much higher than average as per eligibility criteria for the study. Two different colleges were included for the study, one for the experimental group and one for the control group to prevent contamination in April 2016. The experimental group was selected from Sri Sukhmani College of Nursing and the Control group was selected from Amar Professional College of Nursing, Dyalpura, District Mohali, Punjab. Purposive sampling technique was utilized to select 60 subjects (30 in each group). A survey was done in both the settings among all the nursing students present at the time of data collection to identify the stress level using the Perceived Stress Scale. 103 subjects were surveyed in a setting chosen for the experimental group, 39 subjects had “much higher than average stress”, 28 had “slightly higher than average stress”, 26 had “average stress” and 10 subjects had “slightly lower than average stress”. Out of 39 subjects who had “much higher than average stress”, 30 subjects were conveniently selected in the experimental group. Whereas 88 subjects were surveyed in a setting chosen for the control group, 35 subjects had “much higher than average stress”, 21 had “slightly higher than average stress”, 19 had “average stress” and 13 subjects had “slightly lower than average stress”. None of subjects had “much lower than average stress” in both the settings. Out of 35 subjects who had much higher than average stress, 30 subjects were conveniently selected in control group. Subjects who have much higher than average perceived stress level as measured by Perceived Stress Scale were included in study. Perceived Stress Scale by Sheldon Cohen (1983)\(^9\), the standardized tool was selected to determine the stress level among student nurses. The tool was considered for study after extensive review of literature and experts’ opinion. Tool consisted of 2 sections. Section- A: Demographic profile (It consisted of personal information about the nursing students such as age, gender, course, types of family, family income, residence, living arrangement, and marital status), Section-B: Perceived Stress Scale (This section consisted of 5 point likert scale i.e. Sheldon Cohen’s (1983) Perceived Stress Scale which includes 10 items, out of which 4 are positive statements and 6 are negative statements). Co-efficient alpha reliability of this scale is 0.84. Researcher got the training and certificate from psychologist for laughter therapy. Pre test of all the students was done in both colleges by using Perceived Stress Scale. A written informed consent was taken from each study sample. Laughter exercise sessions had been taken by researcher for experimental group for 15-20 minutes every day for 10 days. Everyday laughter therapy was started by doing deep breathing exercises. Laughter therapy was done in group with techniques greeting laughter, hearty laughter, milkshake laughter, one meter laughter, cell phone laughter, argument laughter and appreciation laughter. Deep breathing exercise was done after every two laughter exercises to relax the participants and it was also done at the end of each session. No laughter therapy was given to the control group. After 10 days, the post-test stress level was conducted among both experimental & control groups using the Perceived Stress Scale, and the researcher thanked the participants for their cooperation & interest during laughter therapy sessions. Approval from the ethical and research committee of Sri Sukhmani College of Nursing was taken before starting the study.

Results

100% subjects were females in both the groups. In experimental & control groups, majority of the subjects (76.66%, 70%) fall under age group of 17-20 years, 100% subjects were females and single and 70%, 63.33% were undergoing B.Sc.(N) course. In the experimental group, 56.66% subjects belonged to nuclear families whereas in the control group 50% each belonged to joint family and nuclear family. In experimental group, 26.66% each had family income every month between Rs.10,000-20,000, Rs.20,001-30,000, and Rs.30,001-40,000 although in control group half of the subjects (53.33%) had family income per month between Rs.10,000-20,000. In the experimental group & control group the majority of subjects (86.66%, 90%) were presently living in the hostel and nearly half of the subjects (50%, 43.33%) belonged to rural areas. For matching of experimental & control group chi-square test was applied for each demographic variable. For all the variables the value of chi-square was identified non-significant at \( p \leq 0.05 \). Hence, both groups were considered homogenous [Table 1].

In the experimental group, the mean post-test stress scores (15.13 ± 1.776) of subjects was less from
control group (22.43 ± 1.9241), to find the difference unpaired t-test was applied, the value of t was 15.270 at df 58 and p = 0.000, which was found to be statistically significant at p level ≤ 0.05. And in control group, the mean pretest stress scores of subjects (22.73 ± 1.9640) was found approximately similar to the mean posttest scores (22.43 ± 1.9241), to find the difference one of the Parametric test was applied i.e. “paired t-test”. The value of t was 0.9312NS at df 29 was identified to be statistically non-significant at p level ≤ 0.05. The mean post-test stress score of subjects in the experimental group (15.13 ± 1.776) was lowest than mean pre-test scores (23.40 ± 1.811), to find the difference “t-test” was used, value of t was 23.262 at df 29 which was identified to be statistically significant at p level ≤ 0.05 [Table 2].

Table 1: Percentage distribution of sample characteristics in experimental and control group

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Experimental group n=30</th>
<th>Control group n=30</th>
<th>Chi square</th>
<th>df</th>
<th>p-value</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<td>16-20</td>
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<td>21</td>
<td>70</td>
<td>0.3409NS</td>
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<td>B.Sc.(N)</td>
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<td>Joint</td>
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<td>43.33</td>
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<td>50</td>
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<td>Nuclear</td>
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<td>56.66</td>
<td>15</td>
<td>50</td>
<td>0.3409NS</td>
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<tr>
<td>Family income</td>
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<td>10,000-20,000</td>
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<td>16</td>
<td>53.33</td>
<td>0.3333NS</td>
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<tr>
<td>20,001-30,000</td>
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<td>26.66</td>
<td>4</td>
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<td>0.3333NS</td>
</tr>
<tr>
<td>30,001-40,000</td>
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<td>13.33</td>
<td>0.3333NS</td>
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<tr>
<td>Above 40,001</td>
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<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rural</td>
<td>15</td>
<td>50</td>
<td>13</td>
<td>43.33</td>
<td>1.0087NS</td>
</tr>
<tr>
<td>Semi-urban</td>
<td>4</td>
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<td>7</td>
<td>23.33</td>
<td>0.0087NS</td>
</tr>
<tr>
<td>urban</td>
<td>11</td>
<td>36.66</td>
<td>10</td>
<td>33.33</td>
<td>0.0087NS</td>
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<tr>
<td>Living arrangement</td>
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<td>100</td>
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Table 2: Comparison of Mean Pre-test and Post-test Stress Scores among Nursing Students in both Groups

<table>
<thead>
<tr>
<th>Pre-Post test</th>
<th>Experimental Group n = 30</th>
<th>Control group n = 30</th>
<th>t-value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± S.D</td>
<td>mean ± S.D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test</td>
<td>23.40 ± 1.811</td>
<td>22.73 ± 1.9640</td>
<td>1.2375NS</td>
<td>58</td>
<td>0.1771</td>
</tr>
<tr>
<td>Post-test</td>
<td>15.13 ± 1.776</td>
<td>22.43 ± 1.9241</td>
<td>15.270*</td>
<td>58</td>
<td>0.000</td>
</tr>
<tr>
<td>t= 23.262*</td>
<td></td>
<td>df= 29</td>
<td>p-value= 0.0001</td>
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</tr>
</tbody>
</table>

In experimental & control group during pre-test all (100%) nursing students had much higher than average stress. During post test in experimental group, 56.66% subjects had average stress, 43.33% had slightly higher than average, none of the subjects had much higher than average stress whereas in control group none of the subjects had average stress, 16.66% students had slightly higher than average, and 83.33% students had much higher than average stress. Figure 1 shows that After the implementation of laughter therapy in experimental group less than half had slightly higher than average stress, more than half had average stress and none of subjects had much higher than average stress whereas in control group where laughter therapy was not administered, majority of subjects had much higher than average stress and very few had slightly higher than average stress.

Table 3 Association of Mean Post-test Stress Scores with their selected demographic variables of experimental group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>mean ± SD</th>
<th>F / t</th>
<th>df</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (In years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16-20</td>
<td>23</td>
<td>15.434 ± 1.804</td>
<td>1.7435NS(t)</td>
<td>28</td>
<td>0.0922</td>
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<tr>
<td>Above 20</td>
<td>7</td>
<td>14.142 ± 1.345</td>
<td>1.2649NS(t)</td>
<td>28</td>
<td>0.7931</td>
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<tr>
<td>Course</td>
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<td></td>
</tr>
<tr>
<td>B.Sc. (N)</td>
<td>21</td>
<td>15.190 ± 1.778</td>
<td>1.6514NS(t)</td>
<td>28</td>
<td>0.1098</td>
</tr>
<tr>
<td>GNM</td>
<td>9</td>
<td>15.000 ± 1.870</td>
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<td></td>
<td></td>
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<tr>
<td>Type of family</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint.</td>
<td>13</td>
<td>14.538 ± 2.145</td>
<td>1.0206NS(F)</td>
<td>3</td>
<td>0.9959</td>
</tr>
<tr>
<td>Nuclear.</td>
<td>17</td>
<td>15.588 ± 1.325</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Family income</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000-20,000</td>
<td>8</td>
<td>15.250 ± 1.669</td>
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<tr>
<td>20,001-30,000</td>
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<td>15.125 ± 1.885</td>
<td>0.2025NS(F)</td>
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<tr>
<td>30,001-40,000</td>
<td>6</td>
<td>15.125 ± 2.295</td>
<td></td>
<td></td>
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<tr>
<td>Above 40,001</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>15</td>
<td>14.933 ± 2.051</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rural</td>
<td>4</td>
<td>15.500 ± 1.732</td>
<td>0.1586NS(t)</td>
<td>28</td>
<td>0.8752</td>
</tr>
<tr>
<td>Semi-urban</td>
<td>11</td>
<td>15.272 ± 1.489</td>
<td></td>
<td></td>
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<tr>
<td>Urban</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living arrangement</td>
<td>26</td>
<td>15.153 ± 1.869</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hostel</td>
<td>4</td>
<td>15.000 ± 1.154</td>
<td>1.0206NS(F)</td>
<td>3</td>
<td>0.9959</td>
</tr>
<tr>
<td>Home</td>
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</tbody>
</table>
Table 3 depicts that using ANOVA and t test, in experimental group no significant correlation was identified among post test stress scores and demographic variables, age (t=1.7435), course (t=0.1649), type of family (t=1.6514), family income (F=0.0206), residence (F=0.2025), and living arrangement (t=0.1586) at p level ≤ 0.05. Hence, it can be inferred that the post test stress scores in experimental group was not associated with any of the demographic variables of nursing students.

Table 4 Association of mean post-test stress scores with selected demographic variables of control group.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>mean ± SD</th>
<th>F / t</th>
<th>df</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (In years)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>16-20</td>
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<td>22.238</td>
<td>1.640</td>
<td>0.8447NS(t)</td>
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<tr>
<td>Above 20</td>
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<td>22.889</td>
<td>2.522</td>
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<tr>
<td>Course</td>
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<td></td>
</tr>
<tr>
<td>B.Sc. (N)</td>
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<tr>
<td>Joint.</td>
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<td>22.800</td>
<td>1.971</td>
<td>1.0454NS(t)</td>
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<td>Nuclear.</td>
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<td>22.066</td>
<td>1.869</td>
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<tr>
<td>Family income</td>
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<td></td>
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<td>22.000</td>
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<td>1.1540NS(F)</td>
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<td>Residence</td>
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<td></td>
</tr>
<tr>
<td>Rural</td>
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<td>3.017</td>
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<td>1.902</td>
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<td>0.966</td>
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<tr>
<td>Living arrangement</td>
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<tr>
<td>Hostel</td>
<td>27</td>
<td>22.333</td>
<td>1.961</td>
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</tr>
<tr>
<td>Home</td>
<td>3</td>
<td>23.333</td>
<td>1.527</td>
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</table>

Table 4 depicts that using ANOVA and t-test, in control group no significant correlation was identified in between post-test stress scores and demographic variables, age (t=0.8447), course (t=1.1414), type of family (t=1.0454), family income (F=1.1540), residence (F=0.6738) and living arrangement (t=0.8499) at p level ≤ 0.05. Hence, it can be inferred that the post-test scores in Control group was not associated with any of the demographic variables of nursing students.

Discussion

These findings are supported by the findings of the studies conducted by Karabacak U (2012)\textsuperscript{10} which concluded that majority i.e. 38 (74.50%) of subjects had higher stress and 13 (25.49) of them had low stress. Another study conducted by Sheu S, Lin HS, Hwang SL (2002)\textsuperscript{11} revealed that majority of nursing students 428 (76%) has higher stress, 133 (23.07%) had moderate stress; the most documented stressors were inadequate knowledge and skills, caring for patients, and assignment burden.

In this study laughter therapy was administered and it was advantageous in lowering stress among the nursing students which is supported by the findings of studies conducted by Scott E (2009)\textsuperscript{12} which concluded that after laughter therapy programme total mean score decreased from 5.25 ± 2.01 to 3.02 ± 1.02, the findings of this research study provide evidence that laughter therapy is effective in reducing stress. Berk AR (2005)\textsuperscript{13}, Seaward BL (2003)\textsuperscript{14} which revealed that after laughter therapy in post test 76% subjects had mild stress and 24% had moderate stress, the distinction between pre
and post test stress scores was significant at p < 0.05 level. It was found that, no relationship exist between post test stress level between nursing students and their selected demographic variables in both the groups. These findings are supported by the findings of studies performed by Nicolas AK, Rod A Martin (2010)\textsuperscript{15}, Rahul M (2010)\textsuperscript{16} which concluded that there was no considerable correlation within post-test stress level with their selected demographic variables of nursing students.

Conclusions

Our quasi-experimental study reveals that the pre-test stress scores in both groups were approximately similar. However,

- In Experimental group, mean post-test stress scores were less as compared to mean pre-test stress scores after implementation of laughter therapy.
- In Control group, the mean pre and post-test stress scores were approximately similar. Hence, it is concluded that stress level among nursing students is reduced by use of laughter therapy.

Therefore, the research hypothesis is accepted and it is identified that no correlation was found between the post-test stress scores of student nurses and their selected demographic variables in both groups. (Experimental & Control)

Financial support & sponsorship: Nil

Conflicts of Interest: There are no conflicts of interest.

Ethical Clearances: Taken from the ethical and research committee of Sri Sukhmani College of Nursing, Derabassi, Punjab

References
Type of article: Original article

Current Trends of Poisoning in Tertiary Care Hospitals Located in a Rural Area of Salem, Tamil Nadu, India

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¹Assistant Professor, ²Associate Professor, Department of Forensic Medicine, Annapoorna Medical College and Hospital, Salem, Tamilnadu, India

Abstract

Time wise epidemiological studies are important to find out the trends of poisoning in each region. The present study was conducted by collecting data regarding age, sex, marital status, religion, occupation, type and manner of poisoning of cases which got admitted and treated over a period of 3 years to know the current pattern of poisoning in tertiary care hospitals located in a rural area of Salem, Tamil Nadu.

Total number cases studied were 170 cases, Among them 60 cases (35.3%) belonged to 21-30 yrs, males were 86 cases (50.6%) and females 84 cases (49.4%), both the sex were almost equally distributed, 103 cases (60.6%) were married, 151 cases (88.8%) came from rural area, 160 cases (94.1%) were suicidal and all belonged to Hindu religion. Occupation-wise 56 cases (32.9%) were house wives, students 51 cases (31.8%), followed by farmers, laborers and others. Coming to the type of poisons, maximum were organophosphorous compounds 38 cases (22.4%), followed by Paraquat, 3% yellow phosphorous, oleander seeds and others.

Keywords- Poisoning, epidemiology, age, Organophosphorus

Introduction

Any substances which make life better, if not used wisely and with proper care can become dangerous to human life and those substances can be termed as poisons¹. A poison can be any substance that can harm our body when ingested, inhaled, injected, or absorbed through the skin. The history of poison stretches as early as 4500 BC to the day it was discovered in ancient times and was mainly used by the ancient tribes and civilizations as a hunting tool to kill their prey and enemies².

According to WHO (World Health Organization) data of 2012, approximately 193,460 people died due to unintentional poisoning, 84% of which occurred in low and middle income countries³. The incidence of poisoning in India is among the highest in the world and is estimated that more than 50,000 people die every year from toxic exposure in India which is common cause of morbidity and mortality. This figure could be just the tip of the iceberg and most cases of poisoning actually go unreported especially in third world countries⁴. About 99% of these deaths occur in developing countries like India. Over the last few decades agricultural pesticides have been commonly used for intentional self-poisoning due to their easy availability⁵.

Organophosphorus poisoning is very common mainly in farmers in southern India due to its easy availability and parathion being frequently used. Thus, due to the easy accessibility of these compounds, a large number of suicidal cases are encountered ⁶. In addition to that, snake bites are acute emergencies in tropical and subtropical countries with heavy rainfall and humid climate⁷.

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Along with these various household poisons, plant toxins, drugs and other miscellaneous agents are being used for poisoning. This study is carried out with above mentioned interest and also to know incidence of poisoning which varies from place to place. Therefore it is very important to perform regional studies periodically to identify the extent and evolution of the problem. By this study we can make out the commonest poisons encountered, types of poisoning, and nature of poisoning and socio-demographics in and around Salem. The resulting evaluation will be instrumental in prevention and management of future cases along with providing proper education to all.

Materials and Methods

The present study was conducted at Annapoorna Medical College & Hospital, Salem, Tamil Nadu, after obtaining approval from the institutional Ethics committee. Data of poisoning cases that were brought to the casualty and got admitted and treated over a period of 3 years, 2016-2018 was collected after obtaining permission from the Tertiary Care Centers attached to Vinayaka Mission’s Research Foundation, Salem. The patients included in the study are those who had undergone exposure to poison either by household or agricultural pesticides, stings, snake bites, industrial toxins, toxic plants, drugs or other miscellaneous products. Cases brought dead and died in the Casualty are excluded from the study. The following data regarding type, nature of poisoning, Socio-demographics were collected from hospital records. The obtained data were entered into Microsoft excel and SPSS version 23.0. Descriptive statistics and frequencies were used for data presentation.

Results

The data of 170 admitted and treated poisoning cases over a period of 3 years was collected from May 2016 to May 2018. Among these cases individuals belonging to age group 21-30 are 60 cases (35.3%) which are the maximum. The number of cases above 50 yrs and below 10 yrs were only 9 cases (5.3%) and 4 cases (2.4%) respectively (Table-1) which indicates most of the cases belong to young adults. Coming to sex distribution, males are 86 cases (50.6%) and females 84 cases (49.4%), males are on higher side. 103 cases (60.6%) are married and 151 cases (88.8%) came from rural area. Out of 170 cases a total of 160 cases (94.1%) were suicidal 8 cases (4.7%) were accidental (Table-2). If we look into the occupation 56 cases (32.9%) were housewives, students 51 cases (31.8%), followed by farmers, laborers and others (Table-3). All the 170 cases (100%) belonged to Hindu religion. Coming to the type of poisons, maximum were organophosphorous compounds 38 cases (22.4%), followed by Paraquat, 3% yellow phosphorous, oleander seeds and others (Table-4)

<table>
<thead>
<tr>
<th>Age group in years</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
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<td>2.4</td>
<td>2.4</td>
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<tr>
<td>11-20</td>
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<td>18.8</td>
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<td>41-50</td>
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<tr>
<td>Total</td>
<td>170</td>
<td>100.0</td>
<td>100.0</td>
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</table>
Table-2- Manner wise distribution of cases

<table>
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<th>Manner</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
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</thead>
<tbody>
<tr>
<td>Suicidal</td>
<td>160</td>
<td>94.1</td>
<td>94.1</td>
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<tr>
<td>Accidental</td>
<td>8</td>
<td>4.7</td>
<td>4.7</td>
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<tr>
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<tr>
<td>Total</td>
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Table-3- Occupation wise distribution of cases

<table>
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<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
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</thead>
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<td>2.9</td>
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<td>Driver</td>
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<td>Farmer</td>
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<tr>
<td>HW</td>
<td>56</td>
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<td>32.9</td>
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<td>Labourers</td>
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<td>10.6</td>
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<tr>
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Table-4- Type of poison

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<thead>
<tr>
<th>Type of poison</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC</td>
<td>38</td>
<td>22.4</td>
<td>22.4</td>
</tr>
<tr>
<td>Paraquat</td>
<td>34</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>3% yellow phosphorus</td>
<td>33</td>
<td>19.2</td>
<td>19.2</td>
</tr>
<tr>
<td>Oleander seeds</td>
<td>13</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Cow dung powder</td>
<td>8</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Aluminium phosphide</td>
<td>5</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Glyphosate(herbicide)</td>
<td>4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Imidacloprid (insecticide-neurotoxin)</td>
<td>3</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Cypermethrine(pyrethroid)</td>
<td>3</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Type of Poison</td>
<td>Count</td>
<td>Percentage (%)</td>
<td>Code</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------</td>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbolic acid</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Metformin</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Oduvanthalai</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Snake bite</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Scorpion Sting</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Clonazepam-10 tabs</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Lysol</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Ant powder- cybernmemtcin</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Sedatives-14 tabs, Antihypertensives-nebevelol</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Humic acid and amino acid</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Sodium Acifluorfen (herbicide) with alcohol</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Tab.oxytol overdose( anticonvulsant)-600mg 8tabs, lamitol-50mg 8 tabs</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Hair dye</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Nutmeg- 4 seeds</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Savlon-chlorhexidine+citremide</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Thyroxine- tabs</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Zincphosphide</td>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>170</strong></td>
<td><strong>100.0</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Discussion

Poisoning cases are one of the major socioeconomic burdens on the community. Trends of poisoning vary from place to place and needs to be addressed periodically. Although there is tremendous progress in the field of medicine, poisoning is considered to be one of the major causes of morbidity and mortality in India. In this study, maximum number of poisoning cases was seen in the age group between 21-30 years followed by 11-20 years and less in extreme ages. Similar findings were observed in previous studies. This can be described by the fact that young people put up with stress, financial problems, unemployment, marital conflicts, failure in love etc. Poisoning cases were more in males than females which were also observed by previous studies. This is because of high exposure of men to various agrochemicals and occupational hazards at work. But in studies, done in other countries showed female predominance. With respect to marital status, were married and were unmarried which was similar to the study. Majority of the poisoning cases were observed in rural area, because of its easy availability, lack of immediate medical care. Other studies have reported similar reports. But study conducted by showed poisoning cases were more seen in urban areas as compared to rural areas. Coming to the religion, all the victims were Hindus which were almost similar to the study conducted by where most of the victims were Hindus followed by Christians and Muslims. This is probably because Hindus are the majority of population in India. With regard to manner of death most of the cases were suicidal than followed by Accidental cases which were similar to other studies. This signifies committing suicide by poisoning is easiest method because of easy availability of various agrochemicals and other substances. In this present study poisoning is seen mainly in housewives followed by students and farmers. But in study carried out by Prashant Gupta et al states more poisoning cases in farmers followed by housewives and students. This increasing trend of poisoning in housewives indicates that they are under lot of psychological stress because of family conflicts and other related issues. In this study the commonest poisoning encountered was the organophosphorous compounds, followed by Paraquat and 3% yellow phosphorous. This was consistent with the observations made in previous studies where Organophosphorous compound is the main poison consumed. In the present study, we observed Herbicide Paraquat as second highest poison consumed, this signifies the awareness of people about this highly toxic compound which has no antidote and high fatality rate.

Conclusion

The younger generation of both sexes happens to be affected by poisoning every year and the incidence is increasing day by day. Though there are restrictions for sales of agrochemicals and drugs, the susceptibility to these poisonous substances should not be overlooked. So it is very much important to bring out rapid changes and further strengthen the laws regarding sale of agrochemicals and drugs. This will definitely reduce the misuse of these harmful substances by general public. Farmers who frequently handle agrochemicals should be properly educated and trained regarding safety measures. All hospitals mainly in rural areas should be well equipped to treat poison cases. Hospitals should have mandatory access with poison information centers to get relevant information of the particular poison for prompt treatment. Focus should be given for psychological counseling of patients to prevent suicide by poisoning. Periodically the policy makers should review and implemented poison prevention measures.

Conflict of Interest: No conflict of interest

Funding: No source of funding from any agency

Ethical Clearance: Obtained from institutional ethical committee

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Epidemiological Profile of Burn Cases among autopsies Conducted in Dept. of FMT, RIMS, Ranchi

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Abstract

Introduction: Every year, it was found that burns caused by fire were responsible for about 265,000 deaths globally. More than 90% of fatal fire-related burns occur in developing or lower and middle income countries (LMICs). Out of this, South-East Asia alone accounts for more than half of these fire-related deaths. In South East Asian region, death due to Burns in girl / women is more than that of Tuberculosis, HIV/AIDS and malaria combined. In India, burn injury is found to be one of the major causes of death, especially in females. The problem of death due to burns in developing countries like India is primarily due to various socio-cultural factors prevalent in the country. Some of these factors include poor housing conditions, poor maintenance of electrical appliances, and customs of wearing dresses like sarees or dupatta, practice of dowry, illiteracy level and poverty.

Material and Method: Materials for the present study were collected from the medico legal autopsies of 296 burn cases, performed at the mortuary of Rajendra Institute of Medical Sciences (RIMS), Ranchi, during the period from January 2018 to December 2018. A detailed performa was designed with a definite set of questionnaire which formed the basis of this study.

Results: In our study, out of the 296 cases, the most common age group involved was 21-30 years with a female sex predilection (female to male ratio of 1.5:1). As far as the marital status is concerned, most cases were of married men and women (66.22%) involving mostly Housewives (43.25%). The most common time period involved was between 4 PM to 10 PM (35.47%). Most of the cases took place at home (75.34%).

Conclusion: Our study primarily focuses on the epidemiological profile of data involving burn cases in this part of India which is relatively a backward area comprising of a majority of tribal population.

Keywords - Burn, female, young, married, housewives

Introduction

Fire has been known to mankind for about 400,000 years. Most of the communities believe that the whole universe is made up of five essential elements. Water (Jal), Air (Vayu), Earth (Prithvi), Sky (Aakash) and Fire (Agni). So, this way fire or burns have great importance in our life. The use of fire in various aspects has not only added to our comforts but also added to our misuses by increasing the risk of burns. Fire can be considered as man’s first double-edged sword, evidenced throughout history; it has served as well as destroyed mankind1. Burn injuries are dry thermal injury caused due to contact with raw heat such as flame, radiant heat or some heated solid substance like metal or glass, to the body surface2. Mammalian tissue can survive only within a relatively within narrow range of temperature, 22-440

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Thus burning usually occurs due to contact with flame it may be caused due to contact with hot metal or any other hot solid or hot liquid. The severity of burning very extremely depending on the degree of heat, period of exposure, intensity of heat and age of the person. Burn injury of the skin is characterized by the damage to skin tissue from hot (scald, flash, flame, contact), cold, electrical, chemical, radiation, sunlight, or other sources. Burns constitute one of the most common causes of morbidity and mortality worldwide. They can result in significant disfigurement, physical impairment, work loss, psychological problems, and considerable economic burden. Prevention of burn is considered the best strategy to reduce the overall burden of burns. The impact and the management of burn injury depend on the severity of burn. Although minor burns can be treated at outpatient clinics, the management of patients with severe burns requires multidisciplinary approach in specialized burn care centers. The local treatment of burn wound should address the major concerns of wound care including anti-inflammatory treatment, wound coverage, and prevention of infection and scar formation. Although superficial burns may be managed with topical treatment, deep burns require excision and grafting. The major challenges for treating physicians are to control the infection to avoid development of sepsicaemia and its related complications. Burn injuries and their sequel pose a public health problem. Every year, it was found that burns caused by fire were responsible for about 265,000 deaths globally. More than 90% of fatal fire-related burns occur in developing or lower and middle income countries (LMICs). Out of this, South-East Asia alone accounts for more than half of these fire-related deaths. In South East Asian region, death due to Burns in girl / women is more than that of Tuberculosis, HIV/AIDS and malaria combined. In India, burn injury is found to be one of the major causes of death, especially in females. The problem of death due to burns in developing countries like India is primarily due to various socio-cultural factors prevalent in the country. Some of these factors include poor housing conditions, poor maintenance of electrical appliances, and customs of wearing dresses like sarees or dupatta, practice of dowry, illiteracy level and poverty. The precise numbers of burns incidence is very much difficult to arrive due to large population and lack of incident reporting. The loads of over-population, illiteracy, low socio-economic status, poor standards of safety at home and at work place, corruption etc. has caused a significant rise in burns cases.

The aims and objective of this research work is to study the epidemiology of burns, especially in married women in this region of the country and find out certain reasons and causes of burns and its related deaths.

**Materials and Methodology**

The present study was carried out in the Department of Forensic Medicine & Toxicology, Rajendra Institute of Medical Sciences, Ranchi for a period of one and half year from January, 2018 to December, 2018. The materials for the present study were dead body brought for medicolegal autopsy from various police stations of Ranchi District (Jharkhand) at the Forensic Medicine Department of RIMS, Ranchi. During the study period total 3440 cases were autopsied, out of which only 296 cases where death were due to burns. The information regarding the age, gender, socio-demographic, manner of burns, time of incidence, place of incidence, occupation, etc were gathered from the police papers like inquest report, dead body challan etc, and through detailed interviews of the relatives, neighbours, friends, and police officials accompanying the dead bodies. In case of hospital deaths, hospital papers were also examined. A predesigned tested questionnaire (enclosed as annexure- I) was used to record the information.

Before proceeding with the dissection of the body, external examination of the whole body was carried out very carefully and minutely. The general build of the body was noted and its age, sex verified. The different regions of the body were carefully inspected, one by one and details of the injuries present on them were noted. As routine, a detailed and thorough postmortem examination was carried on every case. To make the study more systematic and error free, a Performa was designed and tested to record detailed observation of post-mortem examination including other relevant detailed information. The findings noted were carefully compiled, analyzed and tabulated. The results were presented in form of tables.

**Inclusion criteria:**

1. All cases of deaths due to Burns coming for post
mortem.

2. Cases considered for study will include subjects of all age group of all genders.

Exclusion criteria:

1. Highly decomposed bodies.
2. Cases of Postmortem Burns.

Results

The present study was conducted in the Department of Forensic Medicine & Toxicology, Rajendra Institute of Medical Sciences, Ranchi for a period of one year from January 2018 to December 2018. Total 3440 cases were autopsied during the period of study, out of which 296 cases were of death due to burns. This comprised 8.60% of the total post-mortem conducted in the department during the study period.

The observations on various aspects were recorded and are being presented here in form of various tables.

1. Age wise distribution:

<table>
<thead>
<tr>
<th>Age groups (in years)</th>
<th>No. Of Cases (N)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 10</td>
<td>24</td>
<td>8.01</td>
</tr>
<tr>
<td>11 to 20</td>
<td>84</td>
<td>28.37</td>
</tr>
<tr>
<td>21 to 30</td>
<td>115</td>
<td>38.85</td>
</tr>
<tr>
<td>31 to 40</td>
<td>48</td>
<td>16.21</td>
</tr>
<tr>
<td>41 to 50</td>
<td>14</td>
<td>4.72</td>
</tr>
<tr>
<td>&gt;50</td>
<td>11</td>
<td>3.71</td>
</tr>
<tr>
<td>Total</td>
<td>296</td>
<td>100</td>
</tr>
</tbody>
</table>

2. Distribution based on Gender:

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of cases (N)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>117</td>
<td>39.53</td>
</tr>
<tr>
<td>Female</td>
<td>179</td>
<td>60.47</td>
</tr>
<tr>
<td>Total</td>
<td>296</td>
<td>100</td>
</tr>
</tbody>
</table>

3. Distribution based on Marital Status:

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>No. Of Cases (N)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>196</td>
<td>66.22</td>
</tr>
<tr>
<td>Unmarried</td>
<td>99</td>
<td>33.45</td>
</tr>
<tr>
<td>Widow</td>
<td>01</td>
<td>0.33</td>
</tr>
<tr>
<td>TOTAL</td>
<td>296</td>
<td>100</td>
</tr>
</tbody>
</table>
### 4. Occupation Wise Distribution of Cases:

**TABLE IV:**

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number of cases (N)</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housewife</td>
<td>128</td>
<td>43.25</td>
</tr>
<tr>
<td>Labour</td>
<td>78</td>
<td>26.35</td>
</tr>
<tr>
<td>Student</td>
<td>66</td>
<td>22.29</td>
</tr>
<tr>
<td>Unemployed</td>
<td>16</td>
<td>05.40</td>
</tr>
<tr>
<td>Business/Self Employed</td>
<td>05</td>
<td>01.69</td>
</tr>
<tr>
<td>Govt. Employee</td>
<td>03</td>
<td>01.02</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>296</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

### 5. Distribution of cases Based on Place of Incidence:

**TABLE V:**

<table>
<thead>
<tr>
<th>Place of Incidence</th>
<th>No. of Cases (N)</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside Home</td>
<td>223</td>
<td>75.34</td>
</tr>
<tr>
<td>Outside Home</td>
<td>52</td>
<td>17.57</td>
</tr>
<tr>
<td>Working Place</td>
<td>21</td>
<td>07.09</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>296</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

### 6. Distribution of cases Based on Time of Incidence:

**TABLE VI:**

<table>
<thead>
<tr>
<th>Time Range</th>
<th>No. of Cases (N)</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>04:01 A.M-10:00 A.M</td>
<td>45</td>
<td>15.21</td>
</tr>
<tr>
<td>10:01 A.M-04:00 P.M</td>
<td>85</td>
<td>28.72</td>
</tr>
<tr>
<td>04:01 P.M-10:00 P.M</td>
<td>105</td>
<td>35.47</td>
</tr>
<tr>
<td>10:01 P.M-04:00 A.M</td>
<td>61</td>
<td>20.60</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>296</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Discussion

There is relative lack of published information about burn injuries in Jharkhand. Present study with a relatively small sample size has revealed pattern of burn injuries among reported cases from a tertiary care hospital of Ranchi, Jharkhand.

The present study was conducted in the Department of Forensic Medicine & Toxicology, Rajendra Institute of Medical Sciences, Ranchi for a period of one year from January 2018 to December 2018. Total 3440 cases were autopsied during the period of study, out of which 296 cases were of death due to burns. This comprised 8.60% of the total post-mortem conducted in the department during the study period.

Age wise distribution:

Most common affected age group in this study was 21–30 years which is consistent with findings of many other epidemiological studies on burn from different part of India like Sawney CP et al\textsuperscript{7}, Ahuja RB et al\textsuperscript{8}, Rai A et al\textsuperscript{9}, Chauhan A et al\textsuperscript{10} and Dalbir Singh et al\textsuperscript{11}.

Gender wise Distribution:

In our study it was observed that the female to male patient ratio was 1.5:1 indicating increased vulnerability of female to burn which is comparable with other findings. A very big sample size (N= 26880) study was conducted at KGMU, Lucknow and reported that female: male ratio 1.4:1\textsuperscript{12}.

The findings of present study is also in concurrence with the other studies done in different regions of India like Kumar P et al\textsuperscript{13}, Lal P et al\textsuperscript{14} and Bhardwaj SD et al\textsuperscript{15}. Earlier studies done by Korah MK et al\textsuperscript{16} and Prasad CS et al\textsuperscript{17} on deceased burn patients from this institute had also reported higher percentage of female victims.

Occupation Wise Distribution of Cases:

Occupation wise distribution of death due to burns revealed that maximum cases belong to housewives in this study. In the studies conducted by Gupta RK and Srivastava AK (1988)\textsuperscript{30}, and Dalbir Singh et al 1997\textsuperscript{31}, similar observation was reported that burns incidence was more common in housewives.

Substantially higher female preponderance in present study might be due to a larger population of Jharkhand resides in rural areas where females are mostly housewives engaged in household works. Unsafe cooking practices like Angithi which uses Koyla, chullas uses wooden and use of kerosene lamps are common in rural areas which expose them to flame burn and kerosene burn. These could be other reasons after intentional injuries due to burn for higher number of female patients reporting to hospital. This higher proportion of female burn admissions is consistent with that of many low- income and middle-income countries such as 53% in Egypt\textsuperscript{18}, 56% in India\textsuperscript{19}, 56% in Iran\textsuperscript{20}, 64% in Sri Lanka\textsuperscript{21} and 67% in Turkey\textsuperscript{22}. Studies from high-income countries report higher proportions in males\textsuperscript{23-26}.

This preponderance of female burns in the current study is likely to be related to the role of women in the family where they take care of cooking, baking and other functions involving heating and cooking equipment. In addition, young females are more likely to be affected by intentional self-harm burns. This interpretation becomes more convincing when we notice that 84% of burns occurred at home and 94% of intentional self harm burns were females.

Distribution based on Marital Status:

Marital status wise distribution of death due to burns revealed that the maximum number of burn victims were married (66.22%) followed by unmarried (33.44%). Least number of cases was from widows.

In the present study, the independent risk factors for intentional self-harm were among female married victims was probably because of the increasing familial stress due to day to day problems like unemployment, illiteracy and poverty, which together give rise to greater issues like marital disharmony and dowry. These results are consistent with the findings of other researchers like Adamo C et al\textsuperscript{27}, Srivastava AK\textsuperscript{28}, and Vaghela Prithwiraj Singh et al\textsuperscript{29} and they were in contradiction to the findings of the studies from other developed countries. The unmarried victims group mostly included men of the adolescent age group and the reasons behind their deaths were rivalry, carelessness at the work place and frustration which arose due to a failure in love/examinations.
Distribution of cases Based on Place of Incidence:

The findings of present study showed that majority of incident of burns occurred at home followed by outside of home and working place. Similar observation was reported by various different authors worldwide. Studies involving admissions for all burns and all ages indicate that the majority of injuries occur at home including 56% in Nigeria, 57% in Turkey, 58% in Israel, and 63% in Norway. In the United States 66% of all hospitalized burns are reported to have occurred at home. In the low-income and middle income countries, the kitchen is the room where burn incidents most commonly occur. Majority of incidences occurred in kitchen. This is because the housewives working in kitchen are more prone to hazards of fire. It was followed by incidences occurred in living room. Most of the suicidal victims prefer closed spaces like living room. This finding is consistent with studies done by Subrahmanyam M and Attia AF et al.

In the studies conducted by Vaghela Prithviraj Singh C et al (2012), Mostafa M.Afify et al (2012) and Shinde A.B. Keoliya A.N. (2013) in other part of India and revealed that burns incidence were more common at home.

Distribution of cases Based on Time of Incidence:

Time of incident wise distribution of death due to burns reveals that maximum numbers of burn cases occurred between 4P.M. to 10 P.M. followed by 10 A.M. to 4 P.M. in my study. In the study conducted by Akhter J.M. and Nerker et al (2010) found that the peak incidence of burn occurred between 5A.M to 10A.M (47.4%) followed by between 11P.M to 4A.M (29.8%) which is not similar to our study. There is no universal division of periods of a day. Hence for sake of convenience, a day was divided in three hourly periods. Maximum number of cases 19 (17.43%) were reported in early evening hours during 18:00 hours to 20:59 hours. This is usual time of cooking by housewives in India. This increases chances of exposure to hazards of fire. Minimum number of cases 7 (6.42%) were reported early night hours during 00:00 hours to 02:59 hours. This finding is consistent with studies conducted by Sharma BR et al and Singh D et al. This is sleeping time for most of the peoples. The cases occurred during this period were due to fall of lamp over bed or body while the victims were asleep. This is not consistent with study done by Akhter JM et al, who observed maximum number of incidences 48.59% in early part of the day followed by 29.13% during evening. This might be due to the fact that Akhter JM et al conducted study in rural part of India which involves majority of agrarian population. They are usually exposed to fire in early morning for cooking, warmth and heating water.

In the study conducted by Mostafa M.Afify et al (2012) observed that majority of the burn incidence occur at night time (53.8%) which is not similar to my study. In the study conducted by Shinde A.B. Keoliya A.N. (2013) found that the peak incidence of occurrence between 10P.M to 06P.M (46.8%) followed by between 06A.M to 2P.M (26.6%) and between 2P.M to 10P.M (26.6%) which is not similar to my study.

Conclusion

- Out of the total 3440 cases autopsied during the period of study, 296 cases involved death due to burns.
- Most common age group affected in this study was 21–30 years closely followed by the age group 11-20 years.
- There was higher preponderance of female cases in this study with a female to male ratio of 1.5:1.
- Maximum number of burn victims were married (66.22%) followed by unmarried (33.45%).
- Burn incidence was seen more commonly in housewives (43.25%) followed by labourers (26.35%) and students (22.92%).
- Majority of incidence of burns occurred at home (75.34%) followed by outside home (17.57%) and working place (7.09%).
- Maximum numbers of burn cases occurred between 4P.M. to 10 P.M. (35.47%) followed by 10 A.M. to 4P.M. (28.72%).

Conflicts of Interest: None

Source of Funding: None

Ethical Clearance: Taken from Institutional Ethics Committee, RIMS, Ranchi
References


Systemic Sclerosis and Pulmonary Tuberculosis Associated with Interstitial Lung Disease: A Case Report

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Abstract

A 59-year-old man had complaints of shortness of breath, thickened and dry skin especially on both hands. Results of thoracic HRCT obtained interstitial lung disease. A year after, the patient underwent geneXpert sputum test because he still complained of coughing with white phlegm and tightness while doing activity. The test obtained Mycobacterium tuberculosis 2+. He received anti-tuberculosis drugs. One month after infection, the patient was evaluated for Mycobacterium tuberculosis, while administration of anti-tuberculosis drugs was continued for up to 8 months. In the second year, the patient had severe restriction, and was given advanced anti-tuberculosis drugs in the sixth month.

Keywords: Systemic sclerosis; interstitial lung disease; tuberculosis

Case Report

A 59-year-old man, a private employee, had a history of smoking >10 years and had stopped since the last 8 years. He had no history of alcohol consumption and autoimmune thyroid disease (ATD) therapy. The patient visited hospital with complaints of thickened and dry skin, especially on both hands (Figure 1), and chest pain during activity. Transthoracic echocardiography and echocardiography results showed left ventricular hypertrophy and mild pulmonary hypertension.

History and physical examination led to suspected SSc. Then, the patient underwent chest X-ray examination (Figure 2). We obtained reticulogranular pattern in the right and left paracardial and cardiomegaly. Acid-fast staining was then carried out on sputum, but we found a negative result.

We performed a chest HRCT X-ray and obtained a reticular pattern image on the posterobasal of the right-left inferior lobe of the lung. These findings supported the characteristics of ILD (Figure 3). Based on the examination results, we conducted an assessment based on 2013 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) criteria for the SSc classification (Table 1). The patient was then diagnosed with SSc and ILD. The...
patient was given with 7 cycles of immunosuppressant cyclophosphamide treatment, followed by cyclosporine for 3 months, then azathioprine for 6 months. The results of pulmonary function examination did not show restriction and obstruction.

One year after being diagnosed, the patient returned to the hospital complaining of a weak body, increased tightness during activity, coughing with white phlegm in the last 1 month, and night sweats. The patient was then hospitalized and underwent treatment for pneumonia. Sputum aerobic cultures showed the presence of Klebsiella pneumoniae. Ceftazidime 1-gram therapy was given every 8 hours intravenously according to the results of antibiotic sensitivity. Chest X-ray evaluation was examined and provided the following results in Figure 4.

GeneXpert results showed medium *Mycobacterium tuberculosis*, while rifampicin resistance was not detected. Examination of acid-fast sputum smear was carried out and the results were 2+. Category 1 tuberculosis treatment was started in this patient with ATD 3 tab 4 FDC.

Two months later the patient came back to the hospital with complaints of shortness of breath while doing activities. Physical examination of the patient showed anaemia in conjunctiva and “velcro” crackles in the lower third of right and left hemithorax. Laboratory tests showed pancytopenia as a side effect of the ATD. Initial management of the patient was nasal oxygenation, cessation of azathioprine and ATD FDC due to pancytopenia. The ATDs given were 750 mg levofloxacin every 24 hours orally, 300 mg INH every 24 hours orally, 1250 mg pyrazinamide every 24 hours orally, and 750 mg ethambutol every 24 hours orally, PRC transfusion 1 flask/day, 4 mg methylprednisolone every 24 hours orally and 5 mg amlodipine every 24 hours orally.

The congestion decreased after 3-day clinical treatment. Thoracic HRCT evaluation showed fibro infiltrates with multiple cavities and tree-in-bud pattern in the right lobe of right and left lungs, tree-in-bud pattern in the inferior lobe of the right lung, cystic bronchiectasis in right lung lobe, bronchial wall dilatation and tram track sign in posterobasal segment of the inferior right-left lung, and multiple enlargement of lymph nodes with the largest size ± 0.6 cm in the right axilla, ±0.5 cm left, and ±0.3 cm in the lower right paratracheal. Sputum aerobic cultures showed the presence of Klebsiella pneumonia (figure 3).

Hb and leukocytes decreased to 8.8 g/dL and 670/uL. Transfusion of PRC 1 flask/day was provided up to Hb=10 gr/dL. Immunosuppressant treatment was restarted with the administration of 180 mg of mycophenolic acid every 24 hours orally. One month after discharge, based on the results of consultations with clinical experts, the patients were given with ATDs 300 mg INH and 750 mg ethambutol for continuation phase for 8 months.

In the sixth month of the continuation phase ATD, the patient stated that complaints of tightness were still present, but the patient could be more comfortable with activities and no coughing. Examination of lung function was carried out and severe restriction was obtained. Phase of the ATD was continued and SSc-ILD treatment was followed-up with mycophenolic acid.
Figure 1. Skin on both hands thickened and dry

Figure 2. Chest X-ray post one month
Figure 3. Chest HRCT in the first year (A) and in the second year (B)

Figure 4. Chest X-ray Just like the chest X-ray of the previous year, reticulogranular pattern of the right and left paracardial, and cardiomegaly, were still visible, but now there was also a pattern of fibroinfiltrates.
Discussion

SSc is a heterogeneous disease and its pathogenesis is marked by 3 things, which are microvascular vasculopathy, autoantibody production, and fibroblast dysfunction resulting in an increase of extracellular matrix depositions. The gold standard to diagnose SSc is not found yet. American College of Rheumatology (ACR) made criteria to classify SSc in 1980 but the sensitivity and specificity were low. These criteria then updated by a joint committee between ACR and European League Against Rheumatism (EULAR) in 2013 with higher sensitivity and specificity (>90%) (3, 4).

Management of SSc includes early diagnose, early diagnose of internal organ involvement, identification of patients with risk of new organ complication, and deterioration of disease related to nonpharmacological therapy. Pharmacological management of SSc includes therapy of involved organ with the recommendation from EULAR (5).

Cyclophosphamide is recommended for ILD related to SSc (SSc-ILD) even though the side effect is quite much, especially in a progressive ILD (recommendation A). Azathioprine orally can also be given as an active therapy in SSc-ILD after administration of cyclophosphamide. A randomized controlled trial to compare effectivity of mycophenolate mofetil and cyclophosphamide in a patient with SSc-ILD and the result showed that both are equal in improving forced vital capacity (FVC) but the recommendation for mycophenolate mofetil usage is not made yet (5-7).

The pathogenesis of SSc is still unclear. Patient with the genetic predisposition of SSc, an external trigger makes the pathologic process start. Triggers of SSc can be a virus/bacteria (pathogen), chemical substance and pollutant (e.g, silica), Human Leucocyte Antigen/HLA (such as B8, DR5, DR3, and DR52), or a phenotype changing of body cells. SSc is a result of 3 related processes which are fibroproliferative vasculopathy of small vessels, abnormality of the innate and adaptive immune system that produce autoantibodies and cell-mediated autoimmunity, and fibroblast dysfunction that cause excessive accumulation of collagen and another extracellular matrix in the skin and internal organs (8).

Endothelial damage is the main process in SSc. Endothelial dysfunction causes the decrease of endothelial NO synthase (eNOS) enzyme production so that the production of basal nitric oxide decrease and lead to vasoconstriction of small vessels in extremities. This process causes hypoxia in peripheral extremities. Innate system immune will produce pro-inflammatory cytokines after exposure to external or internal triggers. Adaptive immunity in SSc is Th2 dominant. The t-cytotoxic cell in a patient with SSc play a role in adding the damage of endothelial with granzyme and Fas/FasL. Debris from the result of the apoptotic cell will become an antigen and trigger B cell to form autoantibody. The antigen in the body also binding with autoantibody, which is then along with cytokines, worsen the damage of endothelial and stimulate fibroblast to deposited collagen (9, 10).

Fibrosis is the end of the pathogenesis of SSc which continues to occur during the life of the patient. Fibroblast of a patient with SSc is having dysfunction due to trigger that continuously causing collagen deposition in skin and internal organs, includes the lung. Accumulation of collagens causes massive fibrosis in lungs and alveolitis which is then called SSc-ILD (9, 10).

There is an immune system dysfunction in a patient with SSc-ILD causing them to be easily triggered by external factors such as viral/bacterial infection, also pollutants that can cause disease manifestations even though only in a small amount (11).

Diagnose of SSc-ILD is accomplished by combining the presence of clinical symptoms, physical examination, pulmonary function test, and radiological finding. The patient can be asymptomatic in the early stage and then complain about dyspnea on effort, unproductive cough, and chest discomfort. In physical examination, “velcro” crackles can be found in auscultation. Pulmonary function test shows a decrease of diffusion capacity (DLCO) and forced vital capacity (FVC). HRCT showed infiltrate or subpleural density starts from the posterior segment of the inferior lobe, interstitial reticular infiltrates, and subpleural honeycombing. Traction and cystic bronchiectasis are the advanced forms of progressive disease course (12).

Indonesia is an endemic country of tuberculosis (TB) so patient with the immunocompromised state is in a very high risk of TB infection. Diagnose of TB is
assessed with the presence of clinical symptom of the respiratory tract which is a productive cough for more than 2 weeks, along with systemic symptoms and confirmed by microbiological and radiological findings. Detection of *Mycobacterium tuberculosis* (MTb) in the acid-fast bacilli staining or molecular rapid test is a gold standard to diagnose TB (13).

The side effect of ATD can be a hematological disorder and immune-mediated pancytopenia is one of them. The underlying mechanism of this emergence is the effect of immunity, interaction with the enzymatic pathway, and direct inhibition of the hematopoiesis system. The incidence of hematologic side effects is reported to be related to the use of rifampicin and also to isoniazid, pyrazinamide, and ethambutol but with a lower incidence than rifampicin (14, 15).

SSC-ILD has a poor prognosis with increasing mortality. The survival rate of patients with severe SSC-ILD is 30% at 9 years. The fastest decrease of pulmonary function and radiological worsening occurs in the first 3 years after the onset of disease (12).

**Conclusion**

Patient with SSC-ILD is susceptible to TB infection and the clinical symptoms of both diseases are very similar, making it hard to be diagnosed. SSC-ILD is a progressive disease and the presence of TB can cause deterioration of the ILD itself, decrease of pulmonary function, and decrease of patient’s quality of life. This case is reported to highlight the importance of early detection of TB in a patient with immune disease and ILD. Appropriate management including the management of side effect is very important to prevent progression and improve the quality of patient’s life.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Acknowledgements:** We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

**Ethical Approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

**Funding:** None

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A Study Protocol for a Randomized Trial on Effect of Safe Patient Handling (SPH) program on Rehabilitation Outcomes and on Safety of Physical Therapists in Rural Hospital

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Abstract

Introduction: Patient management and intervention practices are a crucial component of patient treatment in the hospitals. In recent times the focus is put on early and regular mobilization of patients to boost recovery during and after hospitalization. Increase in inpatient mobilization to improve patient treatment raises a physiotherapist’s physical demands and therefore risk of injury. With the context of a patient mobilization program, better work procedures and decrease in physiotherapist’s injuries while working, SPH program will be incorporated.

Purpose : The goal of this research is to evaluate the effect of SPH program and mobilization in context of efforts to improve quality of health care and safety of patients as well as the physical therapist.

Method : There will be 2 groups, intervention (SPH) and comparison (no-SPH) each group will consist of 20 patients. The intervention group will be consisting the patients admitted in rehabilitation unit for the first three months of the research and the comparison group will consist the patients admitted in rehabilitation unit in the last 3 months of the research. Intervention group will undergo rehabilitation with SPH program which will include equipments like lifts based on ceiling and floors, sit to stand supports, motorized hospital beds and ambulation aids.

Result : Statistical analysis will be conducted. Comparison will be made on a variety of characters such as age, duration of stay and diagnosis to determine equivalence between the groups. Evaluation of the impact of SPH program on recovery outcome will be done by linear regression model. FIM scores of intervention group at admission and discharge will be compared to the comparison group FIM scores at the time of admission and discharge. The linear regression model will represent the graphical overview of the mean mobility scores at the time of admission and at the time of discharge for each group.

Conclusion: The publication of conclusion will be done after conducting the study and obtaining the results through statistical analysis.

Keywords : Safe Patient Handling(SPH) program, physiotherapist, mobilization , moving and lifting.

Introduction

Recent trends in improving patient care in acute settings include early and regular patient mobilization; however, this may add to the physical demands on patient care staff, raising their risk of injury(1). Manual patient management procedures result in repetitive physical loads that can result in injuries in health care settings. SPH program have been introduced in response
to high rates of injury from health care providers\(^{(2)}\). Therapeutic patient handling tasks pose a substantial risk for the therapist to sustain work related musculoskeletal disorders than typical patient handling tasks do because the therapists are exposed for longer amounts of time to high mechanical loads\(^{(3)}\). This program of SPH and mobilization is going to take place in the context of incorporating worker safety procedures associated with patient handling into a wider effort to improve patient care through early and regular patient mobilization and implementing safe equipment and practices for patient handling and mobilization\(^{(1)}\).

Patient management and intervention practices are a crucial component of patient treatment in the hospitals. In recent times the focus is put on early and regular mobilization of patients to boost recovery during and after hospitalization. Increase in inpatient mobilization to improve patient treatment raises a physiotherapist’s physical demands and therefore risk of injury. With the context of a patient mobilization program, better work procedures and decrease in physiotherapist’s injuries while working, SPH program will be incorporated.

Programs for safe patient handling and mobilization of patients are focused on data from biomechanical studies demonstrating that proper biomechanics may not prohibit musculoskeletal disorders which are work related and are caused due to shifting, raising or repositioning the patients. Instead of relying on traditional body mechanics to protect the physical therapists, safe patient handling program provides steps that reduce the weight that the therapist can physically raise and offers guidance on how to use the equipments for mobilization and handling the patients\(^{(4)}\). However S P H program equipment were intended to minimize physical exertion during patient handling tasks and not necessarily to alter the rehabilitation process\(^{(4)}\).

While originally designed to protect health care staff from accidents associated with transporting and treating patient, safe patient handling procedures have been developed as a viable strategy of intervention to improve patient recovery\(^{(4)}\). Rehabilitation services, in particular, are seen to be significantly affected by safe patient handling program. In conventional rehabilitation environment therapist offer patient practical mobility support (e.g., ambulance and transfers), rehabilitating patients to walk and transfer by picking them up and protecting them physically\(^{(2)}\). In addition to increased therapist and patient safety, therapist have observed that S P H programs have increased patient involvement and activities, and therapist were able to mobilize the bariatric and medically complex patients in rehabilitation\(^{(2)}\). Advanced equipment for patient handling is used in rehabilitation settings with SPH programs to help or pick up patients, especially when patients are hefty or dependable\(^{(2)}\). The program will be designed such that it will provide more flexibility to the therapist while working on recovery programs to reduce the risk of overloading\(^{(2)}\).

In view of the number and variety of S P H programs and the fact that they radically modify the patient care, the impact of S P H programs on the functional well being of the patient in recovery needs to be immediately investigated. This work will assess the impact of an safe patient handling program on patient’s functional independence as calculated by Functional Independence Measure (FIM)\(^{(2)}\).

**Objectives**

1. To assess the impact of safe patient handling on recovery of the patient.

2. To observe if there is decrease in patient care worker injuries after incorporating safe patient handling program.

3. The goal of this research is to evaluate the effect of S P H program and mobilization in context of efforts to improve quality of health care and safety of patients as well as the physical therapist.

**Material and Methodology**

The study will be conducted in Ravi Nair Physiotherapy College, after getting the approval from the Institutional Ethics Committee (IEC) of Datta Meghe Institute Of Medical Sciences Deemed to be University, Sawangi (Meghe)

**Study type:** Retrospective cohort study.

**Study setting:** Rehabilitation unit of hospital.

**Study duration:** 6 months.
**Outcome Measure:** Functional Independence Measure (FIM) scale.

Validity of Functional Independence Measure (FIM) – ICC > 0.83.

Reliability of Functional Independence Measure (FIM) - ranges from 0.86 to 0.88.

**Equipment included:**
1. Performing lifts which depends on floor and ceiling
2. Sit to stand assists
4. Ambulation aids.

**Inclusion criteria:**
1. Patient admitted to the hospital for stay of more than 3 days
2. Patients in Out Patient Department (O P D) coming for rehabilitation.
3. Patients with diagnosis of disorder related to musculoskeletal system.

**Exclusion criteria:**
1. Any patient who is critically ill on ventilator
2. Any patient who had a stay of less than 2 days or fewer
3. Any patient who is unwilling to participate.

**Sampling type:** Simple random sampling.

**Sample size:** Twenty in intervention group.
Twenty in non-intervention group.

**Procedure**

Ethical clearance will be obtained from IEC. Data will be obtained from medical records of patients admitted to the hospital, patients will be selected on the basis of their stay in the hospital and diagnosis. Inclusion criteria and exclusion criteria will be implemented. The research will consist of two groups, intervention group (S P H) and comparison group (no-S P H) each group will consist of twenty patients. The intervention group will be consisting the patients admitted in rehabilitation unit for the first three months of the research and the comparison group will consist the patients admitted in rehabilitation unit in the last three months of the research. Intervention group will undergo rehabilitation with S P H program which will include equipments like lifts which depend on floor or ceiling, sit to stand support, motorized hospital beds and ambulation aids.

**Data / Statistical Analysis**

Statistical analysis will be conducted. Comparison will be made on a variety of characters such as age, duration of stay and diagnosis to determine equivalence between the groups. Evaluation of the impact of S P H program on recovery outcome will be done by linear regression model. F I M scores of intervention group at admission and at discharge will be compared to the comparison group F I M scores at the time of admission and at the time of discharge. The linear regression model will represent the graphical overview of the mean mobility scores at the time of admission and at the time of discharge for each group.

**Expected Result**

Considering the high rates of injuries therapists are going through, these services may potentially play a significant role in reducing accidents and at the same time allowing for successful recovery of the patient.

**Discussion**

S P H and mobilization strategy dramatically lowers the workplace injury rates for the physiotherapist(5). Use of mechanical patient lifting equipment has proven to not only avoid musculoskeletal injuries but also reduce the discomfort and damage while manually lifting patients(6). S P H and mobilization also increases the job satisfaction of a health care worker(7). Measurement of the effect of the program is important for clinical management evaluation of the success of recovery services and its long term effect on functioning of the patients(8).

Studies show that the S P H programs results in comparable or even slightly better mobility results for majority of patients. This may alleviate concerns among therapists who fear that S P H programs can lead
to dependency and interfere with functional mobility recovery\(^{(2)}\). Several researches indicate that mechanical patient lifts can help lower the rates of musculoskeletal injuries in therapists\(^{(9)}\).

Less is known concerning the effects of S P H programs on the carrying out of simple everyday tasks (A D Ls), for example dressing or bathing, combing\(^{(4)}\). One of a kind element in the S P H program is embedded and integrated usage of safe handling of the patients and mobilizing equipment for patients and practices in patient care program to improve patient mobility with goals to maintain proper safety of the therapist\(^{(1)}\).

Further research works are required to identify comprehensive guidelines for the S P H program, tailored to variable patient care settings and work requirements\(^{(5)}\). Strategies should be pursued to promote the use of mechanical lifting tools, as fewer amounts of accidents can be expected with its increased use\(^{(10)}\). Aim of the physiotherapy rehabilitation involves maintaining the range of motion and avoiding deformity. It is also necessary to promote active movements to improve trunk control and postural balance\(^{(11)}\). Physical activity and exercise play an significant role to maintain adequate state of health\(^{(12)}\). Positive effects of physiotherapy interventions are gaining trust, confidence of the patient during the rehabilitation process\(^{(13)}\). To prevent differences in the recovery phase between the two groups, recovery time for the both groups should be equal. Evidence of this research will support the patients and the therapists by having a rehabilitation process which is safe and reliable\(^{(14)}\).

**Conclusion:** Recovery does not seem to be hampered by the use of Safe Patient Handling Program. There may be irrational concern among therapists that the use of equipment may result into dependency.

**Ethical Clearance:** The study will be conducted in Ravi Nair Physiotherapy College, after getting the approval from the Institutional Ethics Committee (IEC) of Datta Meghe Institute Of Medical Sciences Deemed to be University, Sawangi (Meghe).

**Source of Funding:** None

**Conflict of Interest:** Nil

**References**


Patient’s Knowledge and Satisfaction Regarding Nursing services and Physical Infrastructure of a Tertiary Care Hospital Situated in Rural area of Northern India (Haryana)

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Abstract

Background: Nowadays health care quality is a universal concern. The health sector is changing rapidly due to the escalating requirements and desires of patients. The most appropriate method to measure a client’s experiences about available hospital services is a patient satisfaction survey. Present study aimed to assess patient’s knowledge and satisfaction regarding nursing services and physical infrastructure.

Methods: 330 patients were selected by purposive sampling technique attending various indoor departments of the selected hospital. Self-structured checklist and three-point rating scale each containing a total 90 sets of items used to assess knowledge and satisfaction. The reliability coefficient for the knowledge tool was 0.87 by KR 20 and satisfaction was found 0.85 by Cronbach’s Alpha.

Result: Study results showed that more than half (59.7 %) patients had very good level of knowledge regarding availability of services. 72.4% & 69.7% of patients were satisfied with Nursing services and physical infrastructure facilities respectively. Item wise distribution of level of satisfaction among patients regarding nursing services reveals that “nurses hear you carefully(64.5%)”, “explain patient’s condition to the family (64.5%),” and “Adequate space in ward (75.5%)” was found ranked 1st with the highest satisfaction, whereas “provide psychological counseling”, “Toilet facility and cleanliness(32.1%)” was found ranked lowest. A significant low positive correlation found between mean knowledge and satisfaction scores regarding nursing services and physical infrastructure as evident by computed ‘r’ value (0.38)

Conclusion: Using these necessary responses from the patients, various shortcomings can be pointed out and notify to hospital administration for improvement of the nursing care services & physical infrastructure.

Key Words: knowledge, satisfaction, Nursing services, Physical Infrastructure, Quality care

Introduction

Satisfaction, like quality, is multifaceted construct¹. Satisfaction of clients premised on multifarious strands in a health care organization. Client’s expectancy and his perception with health care and services are directly congruent². Among all the methods, assessment of client’s satisfaction is the vital for the evaluation of Excellencies in a health care organization. It is important to assess the satisfaction rate of the services which will evaluate whether the services are useful or not³. In the recent epoch, many dimensions together with behaviour of employees, patient staff interaction along with administration issues of the hospital and physical surroundings are crucial aspects of patient satisfaction⁴. A happy patient has higher adherence to treatment protocols and goes for routine follow up for his ill health⁵. Pragmatic evidences confirm that

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majority of health care organizations in India are meagrely involved in the assessment of satisfaction rate. The long queues in the OPD areas, little and unkempt waiting areas, poor condition of bathrooms, unsympathetic approach of health care workers bear articulate testimony to the current fact. Entire considerations make the assessment of patient’s satisfaction with health system even highly necessary. In recent years, patients have become a lot conscious to their rights and cognizant regarding their health. They demand best health care in each facet. Patient’s feels unsatisfied when services received by them are not according to their expectations. Developed countries have many research studies and literatures on satisfaction of patients with health care services but this number is very less in case of developing countries including India. Patient satisfaction with nursing services is highly important to any health care agency as care provided by nurses comprise most health care suppliers because nurses take care of patients for twenty four hours on a daily basis. A German study on the determinants of patient satisfaction in patient care from 2011, by Schoenfelder et al. concluded that satisfaction with nursing services and care has the foremost vital impact on overall satisfaction. Satisfaction assessment will offer valuable and exclusive insights into routine health care in hospital. It is broadly accepted as an autonomous dimension of quality of care as analysis of patient satisfaction includes “internal” (inward-looking) aspects of hospitalization, which frequently stay unrecorded, like communication, warmth or interaction. Satisfaction of patient regarding health care facility situated in a rural area is of vital significance for providing quality services to patients, so keeping this point in consideration; the present research study was conducted to assess patient’s knowledge and satisfaction with nursing services and physical infrastructure in a tertiary care hospital situated in a rural area of Northern India (Haryana).

Material and Methods

Study Design and Study Area:

This was a cross-sectional study based on a descriptive survey design conducted in a tertiary care center situated in the rural part of northern India. The hospital is a center for undergraduate and postgraduate medical teaching and has an operational strength of 940 beds. The hospital has 20 departments and provides outpatient consultations and inpatient services to patients presenting to the hospital from other levels of care or on self-referral. Patients are mainly seen in the Outpatient Department causality unit and special clinics. It receives patients from within Haryana, and the neighboring states of India (Uttar Pradesh, Himachal, Punjab, and Chandigarh). The majority of patients are indigenous Hindu, although the Muslim and Sikh ethnic groups also constitute a substantial proportion of the clientele. Clients with various occupational backgrounds like farmers, private, and govt. service holders, businessmen, etc get the benefit of services present over here.

Ethical consideration was taken from the Maharishi Markandeshwar University institutional ethical committee (under project number 575). Written informed consent was also obtained from all the participants before starting the study Data for the study was collected from clients admitted in medicine, surgery, orthopedics, neurosurgery, and gynecology ward of MMIMS&R hospital Mullana, Ambala, Haryana.

Sample Size and Sampling Technique:

Using a Non-probability Purposive sampling technique a sample size of 330 was taken to detect the level of knowledge and satisfaction among the study participants regarding nursing services and physical infrastructure. However, patients referred or advised for or admitted to the Intensive care unit / cardiac care unit/emergency with conditions related to psychiatry or labour, and those with critical health issues were excluded.

Tools and Technique of Data Collection:

A structured knowledge checklist and rating scale were prepared to assess the knowledge and satisfaction regarding nursing services and physical infrastructure among indoor patients. Both tool consisted of 90 items each divided into 2 parts according to areas of hospital services- nursing services, and physical infrastructure among indoor patients. In knowledge tool each item consist maximum 1 score and minimum score 0. The score obtained by the indoor patient were arbitrarily categorized into four levels- Very good (>75%), Good (61-75%), Average (50-60%), Poor (<50%).
Rating scale for level of satisfaction had a score of 3 points i.e., satisfied score as 3, partially satisfied as 2, dissatisfied as 1. The score obtained by the indoor patient were arbitrarily categorized into three levels: Satisfied (>75%), Partially satisfied (50-75%), Dissatisfied (<50%).

Both tools were validated by 7 experts in the various nursing fields. An interview technique was used to collect the data of the present study.

Reliability of the structured knowledge checklist and Rating scale was computed using Kuder Richardson 20 (KR-20) and Cronbach alpha methods which was found 0.87 and 0.85 respectively. The tool was found valid, reliable, and feasible for the purpose of the study.

Statistical analysis Collected data were entered into Microsoft Excel software and data cleaning was performed. Data were analyzed using SPSS IBM Statistics version 20. Descriptive statistics were generated using mean, standard deviation (SD), frequency, and percentages. Analytical statistics like correlation coefficient and Chi-square test was used to see correlation and association. The value of P < 0.05 was considered statistically significant.

Results

Description of sample characteristics

Frequency and percentage distribution was computed to describe the sample characteristics of the sample. The baseline sample characteristics of the participants showed that 47.9% patients were females and 52.1% were males and as regard to the religion 61.4%, 26.7% patients was Hindu and Muslim respectively, 11.6% was Sikh, and 0.3% belong to Christian religion.

Less than half, (35.2%) patients were having education up to primary school and, (3.6%) were having senior secondary education. Family income of 46.1% patients was Rs <5000 and only 5.5% were having Rs >20,001& above per month. More than half patients (51.5%) were from joint family, and other 48.2% were from nuclear family. As regard to previous hospitalization 52.1% were admitted previously in hospital and 47.9% were not admitted previously and as regard to the type of hospital 32.4% admitted previously in private hospitals previously and 19.7% admitted in government hospitals. More than half (68.8%) have less than 2 visits in hospital previously and, 3.9% has more than 4 visits. Nearly half number of patients, (42.4%) had 1-4 days length of stay and, 9.7% had more than 12 days length of stay in the hospital.

Area wise frequency and percentage of levels of knowledge among indoor patients regarding nursing services and physical infrastructure

More than half (59.7%) (74%) patients were having very good level (>75%), and 12.7% & 2.7 % patients were having below average level (<50%) of knowledge regarding availability of nursing services, and physical infrastructure of hospital respectively.

Frequency and percentage of level of satisfaction regarding nursing services, and physical infrastructure

Findings shows that more than half (72.4%) (69.7%) patients were satisfied with nursing services and physical infrastructure facilities respectively. (as shown in figure 1)
Figure 1: Bar Graph Showing the Percentage of Level of Satisfaction among Indoor Patients Regarding Nursing Services and Physical infrastructure

Item wise distribution of level of satisfaction among patients regarding nursing services

Results reveal the frequency, percentage and rank order of level of satisfaction regarding various Nursing services. Among these, “hear you carefully” and “explain patient’s condition to family” was found 64.5% ranked 1st, “treat you with respect and courtesy” was found 63.3% ranked 2nd. “Provide comfort during nursing care” and “provide psychological counseling” was found ranked lowest as shown in table no. 1

Table No.1: Item Wise Distribution of Level of Satisfaction among Patients Regarding Nursing Services

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Items</th>
<th>Satisfied(3)</th>
<th>Partially satisfied (2)</th>
<th>Dissatisfied (1)</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>During the hospital stay, nurses……….</td>
<td>f</td>
<td>%</td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>2.</td>
<td>hear you carefully.</td>
<td>213</td>
<td>64.5</td>
<td>84</td>
<td>25.5</td>
</tr>
<tr>
<td>3.</td>
<td>treat you with respect and courtesy</td>
<td>209</td>
<td>63.3</td>
<td>88</td>
<td>26.7</td>
</tr>
<tr>
<td>4.</td>
<td>explain you things in a understandable way.</td>
<td>207</td>
<td>62.7</td>
<td>100</td>
<td>30.3</td>
</tr>
<tr>
<td>5.</td>
<td>maintained personal privacy.</td>
<td>208</td>
<td>63</td>
<td>96</td>
<td>29.1</td>
</tr>
<tr>
<td>6.</td>
<td>provide comfort during nursing care.</td>
<td>48</td>
<td>14.5</td>
<td>81</td>
<td>24.5</td>
</tr>
<tr>
<td>7.</td>
<td>discussed care option with you.</td>
<td>193</td>
<td>58.5</td>
<td>81</td>
<td>24.5</td>
</tr>
</tbody>
</table>

N=330
Findings reveal the frequency, percentage and rank order of level of satisfaction regarding Physical infrastructure. “Adequate space in ward” was found 75.5% ranked 1st, “proper electricity supply and ventilation” was found 74.5% ranked 2nd, whereas “Toilet facility and cleanliness” was found 32.1% ranked lowest. (as shown in the table below)

<table>
<thead>
<tr>
<th>Item</th>
<th>Percentage</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate space in ward</td>
<td>75.5%</td>
<td>1st</td>
</tr>
<tr>
<td>Proper electricity supply and ventilation</td>
<td>74.5%</td>
<td>2nd</td>
</tr>
<tr>
<td>Toilet facility and cleanliness</td>
<td>32.1%</td>
<td>13th</td>
</tr>
</tbody>
</table>

Item wise distribution of level of satisfaction among patients regarding physical infrastructure
Table No. 2: Item Wise Distribution of Level of Satisfaction among Patients Regarding Physical infrastructure

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Items</th>
<th>Satisfied(3)</th>
<th>Partially satisfied (2)</th>
<th>Dissatisfied (1)</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>f</td>
<td>%</td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>1.</td>
<td>Adequate space in ward</td>
<td>249</td>
<td>75.5</td>
<td>54</td>
<td>16.4</td>
</tr>
<tr>
<td>2.</td>
<td>Availability of bed side locker</td>
<td>227</td>
<td>68.8</td>
<td>56</td>
<td>17</td>
</tr>
<tr>
<td>3.</td>
<td>Cleanliness in ward</td>
<td>182</td>
<td>55.2</td>
<td>97</td>
<td>29.4</td>
</tr>
<tr>
<td>4.</td>
<td>Temperature maintenance facility in ward according to season</td>
<td>217</td>
<td>65.8</td>
<td>67</td>
<td>20.3</td>
</tr>
<tr>
<td>5.</td>
<td>Quietness and noise free ward during night hours</td>
<td>223</td>
<td>67.6</td>
<td>96</td>
<td>26.1</td>
</tr>
<tr>
<td>6.</td>
<td>Proper electricity supply and ventilation</td>
<td>246</td>
<td>74.5</td>
<td>57</td>
<td>17.3</td>
</tr>
<tr>
<td>7.</td>
<td>Safe drinking water</td>
<td>168</td>
<td>50.9</td>
<td>98</td>
<td>29.7</td>
</tr>
<tr>
<td>8.</td>
<td>Toilet facility and cleanliness</td>
<td>106</td>
<td>32.1</td>
<td>71</td>
<td>21.5</td>
</tr>
<tr>
<td>9.</td>
<td>Sitting arrangement in waiting area</td>
<td>197</td>
<td>59.7</td>
<td>62</td>
<td>18.8</td>
</tr>
<tr>
<td>10.</td>
<td>Availability of dustbins</td>
<td>236</td>
<td>71.5</td>
<td>58</td>
<td>17.6</td>
</tr>
<tr>
<td>11.</td>
<td>Private rooms</td>
<td>174</td>
<td>52.7</td>
<td>68</td>
<td>20.6</td>
</tr>
<tr>
<td>12.</td>
<td>Availability of Curtains for privacy maintenance</td>
<td>240</td>
<td>72.7</td>
<td>55</td>
<td>16.7</td>
</tr>
</tbody>
</table>

A significant low positive correlation was found between mean knowledge and satisfaction scores of indoor patients regarding nursing services and physical infrastructure as evident by computed ‘r’ value of (0.38) as shown in table no. 3.
Table No. 3: Mean Standard Deviation and Correlation Value of Knowledge and Satisfaction Scores of Indoor Patients Regarding Nursing Services and physical infrastructure

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>TEST</th>
<th>MEAN</th>
<th>SD</th>
<th>CO-RELATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Knowledge</td>
<td>73.62</td>
<td>12.98</td>
<td>0.387*</td>
</tr>
<tr>
<td>2.</td>
<td>Satisfaction</td>
<td>221.84</td>
<td>27.96</td>
<td></td>
</tr>
</tbody>
</table>

r = 0.113 at 0.05 level of significance * significant

NS=Non significant

Chi square value showing the association of knowledge score regarding availability of nursing services and physical infrastructure with selected sample characteristics

Results of Chi square association indicates that Type of family (10.47) and present medical conditions (5.99) was found statically significant at 0.05 level which indicate that rate of scoring of knowledge was dependent on type of family and present medical condition, as most of the patient who belonged to joint family have more level of knowledge as they received information from family members and their relatives and the patient with acute illness were more oriented and able to receive information regarding available health care services.

The finding also revealed age, gender, religion, marital status, education, family income per month, number of visits, previous hospitalization was found statistically non significant at 0.05 level.

Chi square value showing the association of satisfaction score regarding availability of nursing Services and hospital infrastructure with selected sample characteristics

A chi square association with level of satisfaction indicates that Religion (20.96), occupation (9.79), and type of hospital (6.48) were found statistically significant at 0.05 levels which indicate that satisfaction was dependent on religion, occupation and type of hospital previously admitted.

The findings also revealed that age, gender, type of family, marital status, education, family income per month, number of visits, previous hospitalization was found statistically non significant at 0.05 level.

Discussion

Results of our study shows that 81.87% patients have overall satisfaction regarding nursing services. In nursing services, “nurses hear carefully” and “explain patient’s condition to family” was found 64.5% ranked 1st, treat patients’ with respect and courtesy was found 63.3% ranked 2nd, maintained personal privacy was found 63% ranked 3rd. These results were found to be consistent with the study conducted by Olowe A Folami F (2019) to assess patient satisfaction with nursing care in selected wards of the Lagos University Teaching Hospital (LUTH). The findings revealed that 77% of the patients showed excellent satisfaction with the quality of nursing care received during their stay on the ward. 43.3% clients explained that nurses explanations were clear about test and treatments, nurses willingness to answer the questions were stated satisfactory by 48.3% patients.49.1% were satisfied by the nurse’s friendly and kind behaviour. More than half (60.8%) stated nurses skills and competencies in very good category but only 12.5% were satisfied with privacy during treatment9.

Findings of our study revealed that for Physical infrastructure.”Adequate space in ward” was found 75.5% ranked 1st, “proper electricity supply and ventilation” was found 74.5% ranked 2nd, whereas “Toilet facility and cleanliness” was found 32.1% ranked lowest. These results were found to be consistent with
the study conducted by Rajkumari B, Nulla P (2017). Nearly one third of the patients, 243 (32.4%) were not satisfied with the cleanliness of the ward. Overall 43.7% patients were poorly satisfied with physical facilities of the hospital. Out of 5 headings under physical facilities, “toilet and its cleanliness” score the least.

References

Medicolegal cases: A Potential Source of Coronavirus (SARS-CoV-2) Infection in Indian Police Personnel

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1 Assistant Professor, Department of Forensic Medicine, 2 Assistant Professor, Department of Anatomy, 3 Research Scholar, Department of Forensic Medicine, 4 Junior Resident, Department of Obstetrics and Gynaecology, 5 Professor, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University

Abstract

India accompanies the world witnessing a rapidly spreading global pandemic of Covid-19. On 24 March, the Government of India ordered a nationwide lockdown that triggered the mass migration of daily wages workers back to their native states. Without prior testing for SARS-CoV-2, it imposed a new challenge towards the authorities to prevent the possible spread of infection. The study aims to determine the infection status of migrants who died in the jurisdiction of Varanasi district during their journey and the possible risk of spread of Covid-19 to frontline workers. The study was conducted between 24 March 2020 and 10 July 2020. A total of 6 cases came to our department who died during travel. The nasopharyngeal and oropharyngeal swabs were taken after the body arrived in the mortuary and sent for detection of SARS-CoV-2 by RT-PCR method to the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology of our Institute. A total of six cases were included in this study, of which 3 were positive for SARS-CoV-2 while others were negative. None of them had symptoms of Covid-19 infection, such as respiratory difficulties, sore throat or fever. The guidelines issued by the Ministry of Health and Family Welfare were limited in scope to hospital deaths and lacked guidelines to be followed by police personnel while investigating unnatural deaths. This brings the police personnel at risk of contracting Covid-19 infection as the cases are getting tested after the arrival at mortuary. The only way of protection is prevention, which mandates laying down the guidelines at the earliest.

Keywords: Covid-19, migrants, medicolegal cases, police, guidelines

Introduction

India accompanies the world witnessing a rapidly spreading global pandemic of Covid-19. On 31 December 2019, the Wuhan health commission reported the first case to the country centre of disease control (CDC) and WHO with pneumonia of unknown diagnosis in Wuhan, China. With the accelerated transmission and mortality of disease, the WHO declared it as a public health emergency of international concern and outbreak on January 30, 2020. On February 11, 2020. Covid-19 was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As the continuous expansion and transmission of diseases continued and affected several countries, the WHO declared Covid-19 as a pandemic on 11 March 2020.

In India, the 1st case of Covid-19 was reported on 30th January 2020, and the first death was reported on 12 March 2020. On 22 March, India observed a 14-hour voluntary public curfew at the request of the government. With the rapid increase in the number of cases, on 24 March, the Government of India ordered a nationwide lockdown for 21 days, affecting the entire 1.3 billion population of the country. With a highly contagious nature of infection and an increase in the
number of cases, on 14 April, the government of India extended the nationwide lockdown until 3 May, which was followed by two-week extensions starting 3 and 17 May with substantial relaxations.  

Since 1 June 2020, the government has started unlocking the country in three unlock phases by dividing the area into different zones on the basis of infection - (i.e. the area). Red, orange and green hot spot and barring containment zones.  

With factories and workplaces shut down and the lockdown imposed on the country, millions of migrant workers were left with no livelihood. They had to deal with income loss, food shortages and unpredictability about their future. Subsequently, hunger and insufficient food stocks became a bigger curse for them and their family. The nationwide restriction ceased daily wage workers at their place with no work and money, and they initiated walking or bicycling hundreds of kilometres back to their home district, with transport restrictions due to the lockdown. The government protection norms of sanitation and social distancing could not be followed by these migrants since they travelled together in large groups.  

Even though later in May the government launched special trains and buses, due to starvation and lack of money, the migrant chose to travel in the cargo compartments of trucks and containers or travel by foot together in large groups ruining the corona protection norms.  

More than 300 deaths were reported until 5 May 2020 due to reasons other than Covid-19 because of unplanned migration of daily wage labourers and marginalized migrants. Eighty deaths were reported on the Shramik Special trains while travelling back home: in a period of a month, they had begun.  

This massive migration without prior testing for SARS-CoV-2 imposed a new challenge towards the authorities to prevent the possible spread of infection.  

The study aims to determine the infection status of those migrants who died in the jurisdiction of Varanasi district during their journey and the possible risk of spread of Covid-19 to the Frontline workers through them.  

### Material and method  

The cases for this study were selected from the dead bodies of migrants brought into the mortuary of our department for medicolegal postmortem examination from the various police stations of Varanasi. The study was conducted between 24 March 2020 and 10 July 2020. A total of 6 cases were reported in our department with death during or after travel.  

History regarding the symptoms before death and circumstances of death were collected from the following sources:  

1. The papers sent by police for medico legal autopsy:  
   (a) Inquest report,  
   (b) Copy of the first information report (F.I.R.),  
   (c) Hospital records/reports if available, etc.  

2. From the interrogation of the concerned personnel –  
   (a) Police constables accompanying the dead bodies, and  
   (b) Attendants/relatives, friends and others of the victim.  

The nasopharyngeal and oropharyngeal swabs were taken after the body arrived at our mortuary and were sent for detection of SARS-CoV-2 infection by RT-PCR to the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology of our Institute.  

### Results  

**Case 1** – A 45-year-old male started walking from New Delhi towards Bihar. He died in Varanasi jurisdiction on 16/04/20. The body was sent for autopsy. Keeping in view the history of travel from New Delhi and the history of tachypnoea before death (mentioned in the inquest report), autopsy was withheld, and swabs were taken on 17/04/2020. Report – negative on 18/04/20. Later, autopsy was conducted, and it was concluded that death was due to starvation.  

**Case 2** – A 26-year-old male coming from New Delhi by motorcycle met an accident on highway in Varanasi
jurisdiction. He immediately fell unconscious and was admitted to the district hospital. He died, and the body was sent for autopsy on 04/05/20. Report – negative on 06/05/20. Later, autopsy was conducted, and it was concluded that death was due to head injury.

**Case 3** - A 37-year-old male coming from New Delhi met an accident on highway in Varanasi jurisdiction. He immediately fell unconscious and was admitted to the trauma centre of our institute. He died, and the body was sent for autopsy on 04/06/20. Report – negative on 04/06/20. Later, autopsy was conducted, and it was concluded that death was due to head injury.

**Case 4** - An 85-year-old male coming from Mumbai by Shramik special train died suddenly on 31/05/2020 during the journey. History taken from relatives revealed 2 episodes of vomiting without any history of fever, sore throat, cough or shortness of breath. There is no evidence of any external injury on the body as per police inquest. Autopsy was withheld, and swabs were taken on 01/06/2020. Report – positive on 01/06/20. The police were informed, and autopsy was waived off.

**Case 5** - An 81-year-old male coming from Mumbai by Mahanagari express train died suddenly on 25/06/2020 during the journey. History taken from relatives reveals that he collapsed suddenly while refilling the water on the platform, but there was no history of fever, sore throat, cough or shortness of breath. There is no evidence of any external injury on the body as per police inquest. Autopsy was withheld, and swabs were taken on 25/06/2020. Report – positive on 27/06/20. The police were informed, and autopsy was waived off.

**Case 6** - A 28-year-old male came from New Delhi to Azamghar (Uttar Pradesh) by personal vehicle on 04/07/2020. He was admitted to the Trauma Centre of our institute on 06/07/2020 with a history of head injury caused by alleged assault. There was no history of fever, sore throat, cough or shortness of breath. Swabs were taken on 07/07/2020. Report – positive on 07/07/2020. He died on 07/07/2020, and the body was sent for postmortem examination. The autopsy was conducted with proper precautions, and the cause of death was injury to the head.

**Discussion**

A total of six cases were included in this study, of which 4 arrived from New Delhi, while the other two arrived from Mumbai. The test reports of the 3 deceased who arrived from New Delhi were ‘negative’, and their autopsy was conducted afterwards. The remaining 3 deceased tested positive, of which 2 had travelled from Mumbai via train and one from New Delhi by personal vehicle. The police and district administration were informed immediately, and autopsy was waived off by the police in case numbers 4 and 5, while autopsy was conducted with proper precautions in case number 6. After being disinfected by 1% sodium hypochlorite solution, all the bodies were handed over to the police in a leakage proof body bag (disinfected by 1% sodium hypochlorite solution on the exterior) for cremation.

**Symptoms of the deceased**

As per the history taken from relatives, co-travellers and police, none of them had symptoms of Covid-19 infection, such as respiratory difficulties, sore throat or fever. Instead, there was a history of vomiting just before death in one (case 4) and sudden collapse of the other (case 5), and both were aged above 80 years. The exact cause of death could not be established in case numbers 4 & 5 as the autopsy was waived off, while the last patient died due to injury to the head.

In case numbers 4 & 5, it is worth noting that both cases did not have typical manifestations of Covid-19. This could mean either they were asymptomatic and died due to some other underlying cause, or they died from Covid-19 with atypical presentations. Most pathological studies are in consensus with the clinical features and clinical course of the disease in general. However, the disease is also known to cause pathological damage to organs such as the heart, liver, kidney, brain, blood vessels and other organs.

Meghan E. Sise et al. (2020) presented a case report titled ‘Case 17-2020: A 68 year old man with Covid-19 and Acute Kidney Injury’ who was finally diagnosed with acute kidney injury and its connection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.13

Carolin Edler et al. (2020) conducted an autopsy - based study titled Dying with SARS-CoV-2 infection—an autopsy study of the first 80 consecutive cases in Hamburg, Germany. Out of 80 cases studied aged
between 52 and 96 years, corresponding to three different categories, 76 cases (95%) were classified as COVID-19 deaths, whereas four deaths (5%) were defined as virus independent non-COVID-19 deaths. Pneumonia accompanied by a fulminant pulmonary artery embolism was observed in eight cases whereas peripheral pulmonary artery embolisms were opined to be the cause in nine cases. Deep vein thrombosis was present in 40% of the cases. As per the author, this is the largest autopsy based study of patients infected by SARS-CoV-2.\(^{14}\)

Andrews et al. conducted a case study titled “First confirmed case of COVID-19 infection in India: A case report” describing a female aged 20 years returned from Wuhan, China on January 23rd 2020. Initially, she only had mild sore throat and rhinitis. She was conscious, oriented and afebrile, with other findings such as pulse rate 76/min, blood pressure 100/70 mmHg, respiratory rate 12/min and oxygen saturation 97 percent in the free atmosphere. General examinations were within normal limits, and she recovered well on symptomatic treatment.\(^{15}\)

Raman Swathy Vaman et al. (2020), in their study titled “A confirmed case of COVID-19 among the first three from Kerala”, India, reported a male medical student aged 23 years studying at Wuhan University, China returned to Kasaragod on 27 January. He developed mild infection in the upper respiratory tract on 30th January. The next day, he was admitted to the District Hospital Kasaragod. On physical observation, the patient was conscious, oriented and afebrile, with no paleness, icterus, cyanosis, clubbing, oedema or lymphadenopathy. Blood pressure was 118/78 mmHg, pulse rate was 88/min and respiratory rate was 14/min. Cardiovascular and respiratory systems were normal. Mild congestion of the throat was reported without any significant enlargement or membrane seen in the tonsil.\(^{16}\)

Lechien, J.R et al. (2020) conducted a study titled Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of coronavirus disease (COVID-19): A multicentre European study investigating the dysfunction in confirmed COVID-19 infected cases concluded that as the disease and the virus both are just out and still evolving. As per the study, the infected patients may present solely with dysfunctions of the olfactory and gustatory systems without other symptoms, and it should be recognized as an important symptom of COVID-19 infection by the international scientific community.\(^{17}\)

J.E. Morley and B. Vellas (2020) in an editorial titled Covid-19 and older adults stated that seriously affected patients with acute respiratory distress syndrome can develop myocardial illness leading to mortality along with kidney and liver disease. They also stated that COVID-19 affects the central nervous system by invading it, which causes a rise in inflammatory cytokines expected to be responsible for delirium. Older people may experience an increase in delirium even without a high fever, which is uncommon in comparatively young people.\(^{18}\)

**Government of India guidelines regarding autopsy of Covid-19 positive medico-legal cases**

As per the available scientific literature to date, the survival of viruses gradually decreases with time in a dead body, but there is no specific time limit to declaring the body noninfective. Therefore, it is advisable to adopt precautions because dead bodies can be a potential source of infection, especially for those who come in contact during the initial period.

The cases of unnatural deaths are medico-legal cases that warrant an investigation into the cause of death by police and autopsy if required. The process is known as police inquest (Section 174, The Code of Criminal Procedure -1973), which includes visiting and examining the crime scene and the deceased by police and request for an autopsy if required. This means police is the first responder if such a death occurs outside the hospital, which makes them vulnerable to contracting the infection.

There is a comprehensive guideline issued by the Ministry of Health and Family Welfare (MoHFW) dated 15/03/2020 titled - ‘Covid-19: Guidelines on Dead Body Management.’ The guidelines were based on the current epidemiological knowledge about the COVID-19. At that time, India had travel-related cases and few cases of local transmission. At that stage, all suspected/confirmed cases were isolated in a health care facility. Hence, the document was limited in scope to hospital deaths.\(^{19}\)
This document was, however, coming up short of a guideline to be followed by police personnel while visiting at a crime scene, investigating, handling and transporting a case of unnatural death. As mentioned above, all the cases were tested for Covid-19 after they arrived at mortuary or hospital. The police, unaware regarding the Covid-19 status of the deceased beforehand, did not follow the standard precautions such as the use of personal protection equipment while investigating, handling and transporting such cases to the mortuary for medico-legal autopsy. This brings the police personnel and all those involved at risk of contracting Covid-19 infection as the cases are getting tested after the arrival at mortuary.

This is happening as India lacks a proper guideline to be followed by police while handling medico-legal cases. With cases increasing exponentially, this is of utmost importance to lay down at the earliest, a guideline for the police in handling the unnatural deaths in medico-legal cases, train them for the same and provide them with sufficient resources to protect themselves from contracting the Covid-19 infection.

The following propositions can be considered while drafting the guidelines:

1. It is in goodwill of the police that Covid-19 testing should be done in every case of unnatural death before starting the inquest, preferably at the site of death rather than waiting for a day or two.

2. If the case is positive, the police must use personal protection equipment (PPE kit) during the investigation and transportation of the body.

3. Autopsy to be done in very selective cases.

**Conclusion**

The current pandemic is still ongoing, and the scientific community is still learning new things about this disease. The only way of protection is prevention, especially in the case of frontline workers, be it healthcare workers or police. This mandates laying down the guidelines at the earliest.

**Acknowledgment:** The Author would like to thank the faculty and staff of the department for their valuable support and help with data collection from autopsied cases.

**Conflict of Interest:** Nil

**Source of Funding:** This research was not financially supported by any funding agencies.

**Ethical Clearance:** The present study was approved by “Institutional Ethical Committee” of Institute of Medical Sciences, Banaras Hindu University, Varanasi.

**References**


Lunar Cycle and its Correlation with Unnatural Death in Different Age Group

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Abstract

It is believed abstraction that the Planets and stars influence human health and psychology is a popular notion in astrological science in India. However, debates are still ongoing about the exact effects and their extent and quality. This study aims to deduce the correlation between unnatural deaths in different age groups and the lunar cycle in 2819 cases. The cases were selected from the dead bodies brought into the mortuary of the department for medico-legal postmortem examination from the various police stations of Varanasi between the duration of 1st January 2013 to 17th July 2014. The compiled data were studied and analyzed statistically by employing the ‘one-way parametric ANOVA.’ It was observed that out of 2819 cases the most affected age group was between 21-40 years (52.4%) followed by the age group 41-60 years (23.1%) and 13-20 years (13.7%). The insignificant rise in unnatural death among different phases of the lunar cycle of different age groups indicates minimal correlation and association with the lunar cycle. A detailed study may be done in the future considering one parameter at a time.

Keywords: Full moon day, New moon day, Unnatural death

Introduction

It is believed abstraction that the Planets and stars influence human health and psychology is a popular notion in astrological science in India. However, debates are still ongoing about the exact effects and their extent and quality.

The moon and paranormal activity have often been associated with each other. The common belief is that a full moon is the most favorable time for paranormal investigations. Others believe the best time to experience paranormal activity is two or three days before or after the full moon and new moon. Why? No one can say for sure. One theory about investigating during a full moon is an increased gravitational pull on the earth from a full moon. This event could cause the ascend in paranormal activity.

C P Thakur, Dilip Sharma (1984) randomly selected 3 police stations of Bihar (India), i.e. Gaya Sadar, Kirtya Nand Nagar, and Sonari. Each at least 300 km apart from another. The number of crimes that occurred during 1978-82 was noted each day. Data were pooled and analyzed by the computer using a basic programming method. It was observed that the incidence of crimes committed on full moon days was notably higher than on any other day. Although a small peak in the number of crimes was observed on new moon days, it was insignificant as compared to those committed on other days¹.

Owen C et al. (1998) carried out a prospective study of lunar cycles and violent behavior. The study established no significant relationship between total violence and aggression or level of violence and aggression and different phases of the moon².
Biermann T et al. (2005) conducted a population-based study to assess the influence of the lunar phases on suicides according to age, sex, and chosen method. The study was conducted in Middle Franconia between 1998 and 2003 for a total of 3054 suicides (1949 males and 1105 females) cases. No significant connection was observed between the full, absent, and moon’s interphases and suicides. Nevertheless, a weak association was present between the no moon days and the inclination of the non-violent methods of suicide in men aged less than the median of 40.2 years. They concluded that there was no relationship between suicide and different phases of the moon.

A similar sort of study titled ‘Suicides and the lunar cycle’ was done by J.M. Gutiérrez-García and F. Tusell (1997) in which the results were no different from Biermann T et al. (1997).

Jay Karan et al. (2010) in their study titled ‘Full Moon Days and Crime: Is there any association?’ found that there was no noteworthy difference in crimes happening on full moon days and other days (p = 0.07). On stratification, there was no difference between full moon days of the week, and the same non-full moon day of the week except on Wednesday.

A study conducted by Joseph A. Schafer et al. (2010) in United States titled ‘Bad moon on the rise? Lunar cycles and incidents of crime’ and a similar study executed by Teresa Biermann et al. (2009) in Germany, titled ‘Relationship between lunar phases and serious crimes of battery: a population-based study’ also concluded that there is no correlation between the lunar cycle and incidence of crime.

Varinder S. Parmar et al. (2014) did a study using three different definitions of a full moon. The result was different in different definitions of a full moon. Therefore, they quoted that the different definitions of “full moon” may relate to the discrepancies in the findings of full moon studies. There is a need for standardization of the definition of the “full moon” for future research.

Mayank Gupta et al. (2015) studied homicidal and suicidal deaths and their correlation with the lunar cycle. The study reveals a rise in incidences of homicide and suicide during the full moon but is statistically insignificant.

**Material and Method**

The cases in the present study were selected from the dead bodies brought into the mortuary of the department of Forensic Medicine, Institute of Medical Sciences, BHU for medico-legal postmortem examination from the various police stations of Varanasi. The total duration of the study was 18.5 months (1st January 2013 to 17th July 2014). A total of 2819 cases were taken in this study, which includes deaths by suicide, homicide, and accidents.

The history regarding the circumstances of the unnatural death and other relevant data were collected from the following source:

1. The papers sent by police for the medico-legal autopsy:
   a. Inquest report,
   b. Copy of the first information report (F.I.R.),
   c. Death certificate if hospital death is there,
   d. Suicidal notes/other relevant reports, etc.
   e. Hospital records/reports if available, etc.

2. From the post-mortem register maintained by our department

The compiled data were studied and analyzed statistically by employing the ‘one-way parametric ANOVA’ and the results of the analysis have been presented in various tables that were discussed and concluded.

**Observations and results**

The study includes 2819 cases and it aims to deduce the correlation between unnatural deaths in different age groups and the lunar cycle. The cases were categorized in the different age groups, i.e. 0-12 years, 13-20 years, 21-40 years, 41-60 years, 60 years onward.

The lunar cycle has been divided into three categories:

1. Full moon days (includes 2 days before and after the full moon event)
2. New moon days (includes 2 days before and after
the no/new moon event)

3. Rest of the days (i.e. excluding 1 and 2)

A total of 19 events of full moon and new moon each occurred during 18 and half months of the study. It was observed that out of the 2819 cases, the age group affected most was between 21-40 years (52.4%) followed by the age group 41-60 years (23.1%) and 13-20 years (13.7%). The extreme of ages, i.e. below 12 years and above 60 years contributes 3.7% and 7.1% respectively to unnatural deaths.

**Table - 1** indicates the rate of incidence of unnatural deaths over the different phases of the lunar cycle. It is observed that the rate is found to be in the following order:

- Full moon days (5.22) > overall rate (5.02) > new moon days (4.98) and the rest of the days (4.98).

The rise in incidences during the full moon days over the others is statistically insignificant as indicated by the P-value (0.712).

**Table - 2** indicates the rate of incidence of unnatural deaths of the children below the age of 12 years over the different phases of the lunar cycle. It is observed that the rate is found to be in the following order:

- Rest of the days (6.42) > overall rate (6.12) > full moon days (5.93) > new moon days (5.50).

The rise in incidences during the rest of the day over the others is statistically insignificant as indicated by the P-value (0.267).

**Table - 3** indicates the rate of incidence of unnatural deaths of the children between the ages of 13-20 years (i.e. adolescent age group) over the different phases of the lunar cycle. It is observed that the rate is found to be in the following order:

- Full moon days (6.27) >> overall rate (5.85) > new moon days (5.84) > rest of the days (5.67).

The rise in incidences during the full moon days over the others is statistically insignificant as indicated by the P-value (0.516).

**Table - 4** indicates the rate of incidence of unnatural deaths in the age group between 21-40 years over the different phases of the lunar cycle. It is observed that the rate is found to be in the following order:

- Full moon days (5.58) > overall rate (5.36) > rest of the days (5.33) > new moon days (5.25).

The rise in incidences during the full moon days over the others is statistically insignificant as indicated by the P-value (0.630).

**Table - 5** indicates the rate of incidence of unnatural deaths in the age group between 41-60 years over the different phases of the lunar cycle. It is observed that the rate is found to be in the following order:

- The full moon days (5.84) > rest of the days (5.71) > overall rate (5.70) new moon days (5.55).

The rise in incidences during the full moon days over the others is statistically insignificant as indicated by the P-value (0.796).

**Table - 6** indicates the rate of incidence of unnatural deaths in the age group above 60 years (i.e. geriatric age group) over the different phases of the lunar cycle. It is observed that the rate is found to be in the following order:

- New moon days (6.56) > full moon days (6.33) > overall rate (6.03) > rest of the days (5.82).

The rise in incidences during the full moon days over the others is statistically insignificant as indicated by the P-value (0.329).

Table 1: Showing the Rate of Unnatural Deaths in Different Phases of Lunar Cycle:

<table>
<thead>
<tr>
<th>Lunar cycle</th>
<th>Total no. of days</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Full moon days</td>
<td>95</td>
<td>5.22</td>
<td>2.726</td>
<td>.280</td>
<td>4.67</td>
</tr>
<tr>
<td>Rest of days</td>
<td>373</td>
<td>4.98</td>
<td>2.542</td>
<td>.132</td>
<td>4.73</td>
</tr>
<tr>
<td>New moon days</td>
<td>95</td>
<td>4.98</td>
<td>2.518</td>
<td>.258</td>
<td>4.47</td>
</tr>
<tr>
<td>Total</td>
<td>563</td>
<td>5.02</td>
<td>2.567</td>
<td>.108</td>
<td>4.81</td>
</tr>
</tbody>
</table>

F value= 0.339  P value= 0.712

Table 2: Showing the Rate of Unnatural Deaths in Age Group 0-12 years:

<table>
<thead>
<tr>
<th>Lunar cycle</th>
<th>Total no. of days</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Full moon days</td>
<td>14</td>
<td>5.93</td>
<td>2.973</td>
<td>.795</td>
<td>4.21</td>
</tr>
<tr>
<td>Rest of days</td>
<td>59</td>
<td>6.42</td>
<td>2.415</td>
<td>.314</td>
<td>5.79</td>
</tr>
<tr>
<td>New moon days</td>
<td>24</td>
<td>5.50</td>
<td>1.865</td>
<td>.381</td>
<td>4.71</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>6.12</td>
<td>2.390</td>
<td>.243</td>
<td>5.64</td>
</tr>
</tbody>
</table>

F value= 1.338  P value= 0.267
**Table 3: Showing the Rate of Unnatural Deaths in Age Group 13-20 years:**

<table>
<thead>
<tr>
<th>Lunar cycle</th>
<th>Total no. of days</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full moon days</td>
<td>41</td>
<td>6.27</td>
<td>2.684</td>
<td>.419</td>
<td>5.42</td>
</tr>
<tr>
<td>Rest of days</td>
<td>184</td>
<td>5.76</td>
<td>2.586</td>
<td>.191</td>
<td>5.38</td>
</tr>
<tr>
<td>New moon days</td>
<td>51</td>
<td>5.84</td>
<td>2.453</td>
<td>.343</td>
<td>5.15</td>
</tr>
<tr>
<td>Total</td>
<td>276</td>
<td>5.85</td>
<td>2.574</td>
<td>.155</td>
<td>5.54</td>
</tr>
</tbody>
</table>

F value = 0.664  P value = 0.516

**Table 4: Showing the Rate of Unnatural Deaths in Age Group 21-40 years:**

<table>
<thead>
<tr>
<th>Lunar cycle</th>
<th>Total no. of days</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full moon days</td>
<td>86</td>
<td>5.58</td>
<td>2.578</td>
<td>.278</td>
<td>5.03</td>
</tr>
<tr>
<td>Rest of days</td>
<td>334</td>
<td>5.33</td>
<td>2.410</td>
<td>.132</td>
<td>5.07</td>
</tr>
<tr>
<td>New moon days</td>
<td>87</td>
<td>5.25</td>
<td>2.368</td>
<td>.254</td>
<td>4.75</td>
</tr>
<tr>
<td>Total</td>
<td>507</td>
<td>5.36</td>
<td>2.430</td>
<td>.108</td>
<td>5.15</td>
</tr>
</tbody>
</table>

F value = 0.462  P value = 0.630

**Table 5: Showing the Rate of Unnatural Deaths in Age Group 41-60 years:**

<table>
<thead>
<tr>
<th>Lunar cycle</th>
<th>Total no. of days</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full moon days</td>
<td>67</td>
<td>5.84</td>
<td>2.717</td>
<td>.332</td>
<td>5.17</td>
</tr>
<tr>
<td>Rest of days</td>
<td>254</td>
<td>5.71</td>
<td>2.429</td>
<td>.152</td>
<td>5.41</td>
</tr>
<tr>
<td>New moon days</td>
<td>66</td>
<td>5.55</td>
<td>2.463</td>
<td>.303</td>
<td>4.94</td>
</tr>
<tr>
<td>Total</td>
<td>387</td>
<td>5.70</td>
<td>2.481</td>
<td>.126</td>
<td>5.45</td>
</tr>
</tbody>
</table>

F value = 0.229  P value = 0.796
Table 6: Showing the Rate of Unnatural Deaths in Age Group above 60 years:

<table>
<thead>
<tr>
<th>Lunar cycle</th>
<th>Total no. of days</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% Confidence Interval for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Full moon days</td>
<td>27</td>
<td>6.33</td>
<td>3.00</td>
<td>.577</td>
<td>5.15</td>
</tr>
<tr>
<td>Rest of days</td>
<td>106</td>
<td>5.82</td>
<td>2.487</td>
<td>.242</td>
<td>5.34</td>
</tr>
<tr>
<td>New moon days</td>
<td>27</td>
<td>6.56</td>
<td>2.309</td>
<td>.444</td>
<td>5.64</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>6.03</td>
<td>2.554</td>
<td>.202</td>
<td>5.63</td>
</tr>
</tbody>
</table>

F value=1.120  P value=0.329

Discussion

To start with the hypothesis, the incidence of unnatural death increases in and around full moon days or new moon days or both. To prove this hypothesis, we compared the ‘mean’ of unnatural deaths overall and also those occurring in different age groups to the lunar cycle.

The overall distribution of victims of unnatural death

When we compared the overall ‘mean’ of unnatural deaths in different phases of the lunar cycle, namely the full moon period, new moon period, and the rest of the day. It is found that there is a rise in the mean incidence of unnatural deaths in the full moon period over the new moon period and also the rest of days.

Jay Karan et al (2010) in their study in Surat, Gujarat reported that there was no significant difference in crime events on full moon days and non-full moon days when compared by the Chi-Square test (p = 0.07). Except on Wednesday, no difference was observed between full moon day and corresponding non-full moon day of the week^{5}.

Joseph A. Schafer et al (2010) using police, astronomy, and weather data from a major southwestern American city, reported that there is no correlation between lunar cycles and rates of reported crime^{6}.

Oderda GM, Klein-Schwartz W (1983) found that a larger proportion of total calls to the center, and unintentional poisoning calls occurred during the full moon period. A significantly higher number of unintentional poisonings occurred during the full moon period^{11}.

The age-wise distribution of victims of unnatural death

Out of 2819 studied cases, the largest number of victims of unnatural death belonged to 21-40 years of age group which constituted more than half of the total victims (52.4%) followed by 23.1% in 41-60 years age group. The study also revealed that the extreme age groups, i.e. up to twelve years (i.e. pediatric age group) and sixty years and above (i.e. geriatric age group) were found to be minimal 3.7% and 7.1% respectively as the victims of unnatural death. The high mortality in the age group of 21-40 years can be due to more outdoor activities, the use of high-speed automobiles, also the adventurous nature of the people of this age group. Also, these victims were found bearing most socially active life and responsibility, so they are much exposed to accidents, rivalry, betrayal, stress, and business/property disputes resulting in higher deaths.

When we compared the ‘mean’ of unnatural deaths during different phases of the lunar cycle in the different age groups separately, it is found that except for the
extremes of ages (i.e. pediatric and geriatric age groups as mentioned), all the other age groups show a rise in the mean incidence of unnatural deaths in the full moon period over new moon period and also the rest of days.

The pediatric age group shows fall during the full moon and new moon periods as compared to the rest of the day. While in the geriatric age group, the rise is in new moon days.

The study shows that this rise in mean value during different phases of the lunar cycle is not consistent in all age groups. It is also found that none of these rises in mean value has got any statistical significance.

Oderda GM, Klein-Schwartz W (1983) found that different phases of the moon had no effect with regard to the victim’s age or sex or treatment location.\(^\text{11}\)

When we compared the ‘mean’ of unnatural deaths during different phases of the lunar cycle in the gender groups separately, it is found that there is a rise in the mean incidence of unnatural deaths in the full moon period over new moon period and also rest of the days in both male and female groups.

The study shows that this rise in mean value during different phases of the lunar cycle, which although is consistent in both the groups, it is also found that none of these rises in mean value has got any statistical significance.

Oderda GM, Klein-Schwartz W (1983) found that different phases of the moon had no effect with regard to the victim’s age or sex or treatment location.\(^\text{11}\)

**Conclusion**

The present study is done over 2819 victims of unnatural deaths, the majority of the victims belonged to 21-40 years of age group (52.4%) and the rise in the unnatural deaths during a particular phase of the lunar cycle among different age groups is not consistent. It is also seen that there is an overall rise in the unnatural deaths in the ‘full moon period’ as compared to the other parts of the lunar cycle. Since no rise in the different parameters separately or overall is found to be statistically significant, the study finally concluded that there might be a correlation between unnatural death and the lunar cycle, but there is no association between them in our preliminary study.

In this study, we considered new moon days along with full moon days, which is not the case with many other studies. Also, we took two days back and forth of the new moon and full moon days, which is again the strength of our study. Although this study is not conclusive, further studies can be carried out in two different ways. Firstly, taking one parameter at a time might throw some more light on the association. Secondly, by including the cases in which incidence doesn’t lead to death as well. This may lead to more specific results, and if any, positive association is found, it will be very beneficial to take the necessary steps to bring it down.

**Acknowledgment:** The Author would like to thank the faculty and staff of the department for their valuable support and help with data collection from autopsied cases.

**Conflict of Interest:** Nil

**Source of Funding:** This research was not financially supported by any funding agencies.

**Ethical Clearance:** The present study was approved by “Institutional Ethical Committee” of Institute of Medical Sciences, Banaras Hindu University, Varanasi.

**References**


Role of High Technology Medical Devices and Its Uses in Patient Care

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¹Research Scholar, ²Assistant Professor, Sri Venkateshwara University, Gajraula, ³Associate Professor, Amity Business School, Amity University, Noida, ⁴Director, Corporate Governance Research Centre, New Delhi, ⁵Director, Krishna Engineering College, Mohan Nagar, Ghaziabad

Abstract

The healthcare sector has a vision of providing the best medical facilities to their patients leading to better health care and minimum of medical errors. The healthcare sector tries to provide the patients access to high quality medical devices, safe and security, effective technology and safe products for their health. Increasing complexity of medical technology and consequences for training and outcome of care. The healthcare sector has created a safe sense of services with enhanced and swift technology which is patient reported outcomes to help the patients rate the quality and working of the hospitals to get a clearer picture. This is possible only with the help of technology and this research paper is a primary data based exploration where techology is in the centre.

Key Words: Health care, Technology, Safe services and outcomes.

Introduction

In the era of technology all industries are opting for technology for development and innovation in their field. Technology in today’s World is being adopted by every industry and is playing an important role in industries and in our lives¹. Like all the industries we can see that technology is taking over the healthcare sector focusing on saving lives. In the field of medical sciences, technological innovations in medical devices are taking place all around the World². Medical technology has a vast field of innovation that is innovation in sustaining lives. The technology innovation can be seen in the areas like biotechnology, Information technology, pharmaceuticals and development of medical devices and equipment’s³. The growth in innovations in healthcare sector can be seen from small innovation of bandages and ankle braces to complex innovations like MRI machines, artificial organs, etc. The innovation in the medical devices has focused on integrating innovations into medicines. The innovation in medical devices with integration of telehealth has also lead to robotic surgeries which focuses on helping doctors to do surgeries with the help of robotics sometimes even without their physical presence⁴. Thus technology is trying to work easy and save more and more life. Medical technology is innovating with intensive growth and research in the area the technology has introduced new equipment’s to hospitals making their work easy and complex surgeries successful, not only this technology in healthcare sector has connected thousands of patients and doctors from all around the world. Increasing complexity of medical technology and consequences for training and outcome of care⁵.

Study conducted by Yan Wei, Hao Yu, Zude Guo, on the topic “Hospital efficiency and utilization of high-end technology medical equipment”. In this paper the researcher focused on the relationship between the hospital environmental characteristics and their utilization of high-tech medical equipment⁶. The research focused on the cost and quality of the medical devices used and efficiency of the hospitals in adopting the devices. The researcher found that the use of Computed Tomography(CT) and Magnetic resonance imaging(MRI) in hospitals have increased with hospital cost inefficiency⁷. The researcher aimed to understand the adoption of new medical devices by hospitals and their cost controlling activities in hospitals. The study found out that the relationship between quality improvement of
devices and cost control is very complex due to high cost of technology adoption. The research found out that the hospitals do a comparison to check the benefits with the cost of using high end devices. The main reason of using these devices are quality improvement and increase in income.

Study conducted by Bupe. G. Mwana, Charles Mbohwa on the topic “An assessment of the effectiveness of equipment maintenance practices in public hospitals”. In the research the researcher has found that the use of medical devices is increasing around the World and so are the complexities with the use of the devices. The aim of the study was to analyze the practices adopted in hospitals to maintain the medical devices to reduce the errors happening due to mishandling of the devices. The study conducted found out the maintenance strategies and challenges faced in the maintenance. The study by the researchers found out that devices failure and unavailability of the devices was high due to faulty schedules for devices, high breakdown in the devices was seen due to shortage of skilled manpower. The shortage of skilled manpower affected the service provided to the patients and lead to poor maintenance of the medical devices, not only the shortage of employees the improper training of the employees leads to mishandling of the devices and causes injuries to the patients. The error usually occurs due to lack of staff training and due to non-maintenance of a proper team to look after the maintenance and repairing of the medical devices. The researcher says that the staffs are not trained about the complexities of the devices that are updated timely.

Research Methodology

The data is collected with the help of structured questionnaire. The data is analyzed with help of the statistical software known as SPSS which has used various statistical methods for the analyzing of the data which will be represented with the help of graphs, charts etc. The analysis of the questionnaire on the topic “Role of high technology medical devices and its uses in patient care: Study in reference to Delhi & NCR Hospitals” where sample size was 201.

Hypothesis Testing for the Study

Hypothesis has been formulated on the basis of objectives framed for the study. Since study is moving around the uses of technology in health care industry. Formulated hypothesis is being testing with the help of t-test analysis has been used, data is normally distributed which indicating that, t-test could be applied.

**H0** – There is no significance relation between, carelessness of nurses causing medical errors and untrained professionals using high end technology causes medical error.

**H1**- There is significance relation between, carelessness of nurses causing medical errors and untrained professionals using high end technology causes medical error.

### Table -1 Paired Sample correlation

<table>
<thead>
<tr>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3698</td>
<td>201</td>
<td>.241</td>
<td>.013</td>
</tr>
<tr>
<td>1.3652</td>
<td>201</td>
<td>1.987</td>
<td>.021</td>
</tr>
</tbody>
</table>

### Table-2: Correlation matrix

<table>
<thead>
<tr>
<th>N</th>
<th>Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>201</td>
<td>.321</td>
<td>.342</td>
</tr>
</tbody>
</table>
Table-3: Paired Differences

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carelessness of nurses</td>
<td>1.0046</td>
<td>1.23410</td>
<td>8.92314</td>
<td>232.123</td>
<td>56.942</td>
<td>-7.000</td>
</tr>
</tbody>
</table>

After analyzing data with SPSS, calculated value of p= .000, since standard value is 0.05. Thus calculated value is less than the standard value. So the researcher fails to accept the null hypothesis i.e. there is significance relation between, carelessness of nurses causing medical errors and untrained professionals using high end technology causes medical error. Which means that carelessness of are due to untrained staff or nurses that uses high technological medical devices and these untrained staff due to carelessness causing medical errors.

**H0** – There is no significance relation between, the use of clinical information to improve patient safety and technological devices that increases patient safety.

**H1**- There is significance relation between, the use of clinical information to improve patient safety and technological devices that increases patient safety.

Table -4 Paired Sample correlation

<table>
<thead>
<tr>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.16</td>
<td>201</td>
<td>.367</td>
<td>.026</td>
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<tr>
<td>3.32</td>
<td>201</td>
<td>1.249</td>
<td>.088</td>
</tr>
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</table>

Table-5, Correlation matrix

<table>
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<tr>
<th>N</th>
<th>Correlation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>201</td>
<td>.510</td>
<td>.258</td>
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</table>
Table-6, Paired Differences

<table>
<thead>
<tr>
<th></th>
<th>Paired Differences</th>
<th></th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>95% Confidence Interval of the Difference</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Pair 1</td>
<td>-1.1250</td>
<td>25.00000</td>
<td>12.50000</td>
<td>-152.28058</td>
<td>-72.71942</td>
</tr>
</tbody>
</table>

After analyzing data with SPSS, calculated value of p= .006, since standard value is 0.05. Thus calculated value is less than the standard value. So the researcher fails to accept the null hypothesis i.e. there is significance relation between, the use of clinical information to improve patient safety and technological devices that increases patient safety. Which means that clinical information system is used for improvement of patient safety and this device increases patient safety in hospitals. Thus clinical information system proves to be a technology that is successful in patient safety thus reducing medical errors.

With the increase in technological devices in healthcare sector it is very important for the staff to understand the complexities and working of the devices. The technology in healthcare sector is innovating every year which has made training a very crucial part of proper implementation of the devices as these devices are not only using high tech mechanisms they are also becoming more and more complex which requires timely training for better safety and quality which will reduce medical errors. The implementing cost of high end technological medical devices are very high as agreed by the respondents on the question, that, whether cost of implementing high technological medical devices are high. The dependence of healthcare sector in technology is increasing with time and this has led to new innovations of devices and has focused for on saving lives by providing better diagnosis, better surgical procedures and improvement in the health of patients.

The devices focus on saving lives and making healthcare a safe sector.

The respondents believe that high technological medical devices increase patient safety as 86.1 percent of respondents agree that devices has turned out to be safer and has particular standards that maintain patient safety at each level. The devices though complex help the healthcare sector in better quality and safety. Medical devices also help in reducing medical errors as agreed by the respondents as 86.6 percent agree that high end technological medical devices are successful in reducing medical errors. Though successful implementation of the devices depends on the staff i.e. then trained staff as agreed by 80.1 percent that is 161 respondents out of 201 agree that if staff is trained the implementation of the devices would be done rightly reducing medical errors.

Suggestions for the study

· Timely training should be given to the staff in healthcare regarding working of the devices

· The implementation of the devices should be on the basis of environment

· Regular maintenance and repairing of the devices to be done to reduce medical errors
Safety standards should be followed by all the hospitals and a standard format for all should be used.

Internal and external audits should be conducted in the sector to keep a check on the machines.

Devices should be up to dated according to international standards.

Only technical staff should be allowed to use the high technological medical devices.

The training should be done in a specified framework with goals set prior to the training of the staff so that the staff is accountable for themselves.

Authors Declaration

Conflict of interest – This is to declare that all of us are authors of the following manuscript titled “Role of high technology medical devices and its uses in patient care” and we hereby agree to the following:

1. All of us the authors have read the manuscript and take responsibility for its contents.

2. We have taken special care towards the language and grammar of the article and certify it is correct to the best of our knowledge.

3. We have no conflict of interest.

4. Similarity index is less than 10 percent.

Source of Funding- Self

Ethical Clearance – NIL

References


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Online Classes during COVID-19 Pandemic: Anxiety, Stress & Depression among University Students

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Abstract

COVID-19 pandemic has not only put people at risk of developing physical illness but also face mental distress. This pandemic disease is impacting almost every sector of economy as well as Indian education sector that includes school, colleges and institutes. As everything is virtual with no physical movement most of the mental health illnesses were triggered rapidly such as Depression, Anxiety and Stress. A cross-sectional descriptive study design was done to assess the level of Depression, Anxiety and Stress in grad students who were undergoing online classes and the population for research study was all students studying in bachelors’ program of universities. The sample size was 159 students and the tool used for the research study was DASS 21. The researcher concluded that majority of students experienced moderate Depression 27(16.98%), Anxiety 27 (16.98%) and stress 23 (14.46%) due to online classes.

Keywords: Online classes, COVID-19 Pandemic, Depression, Anxiety, Stress.

Introduction

COVID-19 pandemic has not only put people at risk of developing physical illness but also face mental distress.1 Due to current COVID-19 pandemic, life of the people had taken upside down. It has made a huge change in daily routine, life style, eating habits and sleeping pattern of everyone. The new normal education world is learning from home with minimal physical stimulation.2

The students are under the constant fear of contracting the virus or loosing their loved ones due to this deadly virus. The fear of this pandemic disease is increasing day by day which leads to harmful effects both at the individual level and societal level.3

To combat with COVID-19 a stress management guide, “Doing what matter in time Stress “is given by WHO. This kit contains self-help technique to combat stress. Many states and educational institutions are coming up with tele counseling for mental health services. Still increased cases of Depression, Anxiety and Stress are being reported from various part of the world. Deterioration in mental health may lead to other life-threatening diseases.4

COVID-19 is impacting almost every sector of economy as well as Indian education sector that includes school, colleges and institutes. As everything is virtual with no physical movement and academic stress have led to many most of the mental health disorders. Almost every work is put on hold due to this pandemic disease.5 The large scale industries were closed which drastically affect the economy and all the institutions were closed due to which students all over the world were worried regarding examinations, their future. The teachers are trying their level best to teach students as online classes were conducted on regular basis for the students.6 These online classes lead to various psychological consequences in students.7

To assess these psychological consequences, a study was conducted using a standardized tool DASS 21 which was developed by Lovibond and Lovibond.8 This tool consists of 21 questions to assess Depression, Anxiety and Stress.9 The symptoms of depression are characterized by low self esteem, hopelessness, lack of positive attitude, lack of interest in life, social isolation, sadness, insomnia, feeling of guilt and disturbed...
Anxiety usually occurs with depression. It is a state of fear, apprehension, subjective feeling of anxious affect with autonomic arousal and skeletal muscle tension. Stress is a feeling of emotional, physical tension. The person also experiences irritability, restlessness, impatience.

As there is a complete change in education system, family dynamics and the social life during lockdown students are experiencing various psychological problems. So the prevalence of Depression, anxiety and stress is more among students. The various demographic variables also have great impact on Depression, anxiety and stress level of students. Therefore, the researcher conducted a cross sectional study with the aim to find stress, anxiety and depression in university students who are attended classes online. Also, association between demographic variables and presence of mental health issues was assessed.

**Methodology:**

**Research Design:** A cross-sectional descriptive study design was chosen. 159 students who were attending online classes and willing to participate in study were selected using purposive and snow ball sampling. Informed consent was taken from the study participants to assure anonymity and confidentiality of data. Data was collected using a standardized tool DASS 21. This tool is four-point rating scale which consists 21 items to assess depression, anxiety and stress separately with minimum score of 0 and maximum score of 163. Higher the score in each category and more will be level of ailments such no, mild, moderate, severe and extremely severe. Data was collected from participants from 9/07/20 to 15/07/20. Researcher collects the email id of the students of different streams. Google forms were used to collect the information from students. Google form consists of consent form and structured questionnaire. This form was sent to students and they were asked to circulate the form in their contacts. The students were asked to revert back within a week. Collected data was checked for completeness and analysis was done using SPSS latest version 25.

### Results

**No. of College Students**

<table>
<thead>
<tr>
<th>Depression Level</th>
<th>No. of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>79</td>
</tr>
<tr>
<td>Mild Depression</td>
<td>16</td>
</tr>
<tr>
<td>Moderate Depression</td>
<td>27</td>
</tr>
<tr>
<td>Severe Depression</td>
<td>22</td>
</tr>
<tr>
<td>Extremely Severe</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 1 Level of Depression among college students undergoing online classes during lockdown
The above bar graph shows that majority of students 79 (49.68%) were normal, 16 (10.06%) had mild depression, 27 (16.98%) had experienced moderate depression, 22 (13.83%) had severe depression, 15 (9.43%) were under extremely severe depression.

![No. of College Students](image)

**Figure 2 Level of Anxiety among college students undergoing online classes during lockdown**

Above pie chart shows that majority of students 69 (43.39%) were normal, 20 (12.57%) students had mild anxiety, 27 (16.98%) students experienced moderate anxiety, few students 13 (8.17%) had severe anxiety, 30 (18.86%) students experienced extremely severe anxiety.

![No. of College Students](image)

**Figure 3 Level of Stress among college students undergoing online classes during lockdown**

Above bar graph shows that majority of students 100 (62.89%) were normal, 14 (8.80%) students experienced mild stress, 23 (14.46%) students were under moderate stress, 15 (9.43%) students had severe stress, minority of students 7 (4.40%) experienced extremely severe stress.
Conclusion

This study concluded that online learning has a great impact on mental health of university students and causing stress, depression and anxiety of various levels. Majority of students experienced moderate Depression 27(16.98%), Anxiety 27 (16.98%) and Stress 23 (14.46%) during online classes. More research is needed to find best possible coping strategies to assure mental health and to find out innovative yet effective ways of learning.

Conflict of Interest: Nil.

Ethical Consideration: Ethical permission was taken from ethics review committee of institute.

Source of Funding: Self.

References


Reflection of Stress in the Oral Cavity

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Abstract

Stress is based on the physical, mental, or emotional reaction to events which cause tension in the body or mind. Different types of diseases affect the human body, some of which may have an unknown etiology. It is one such etiology that predisposes many diseases. Dentists often come across patients in their everyday practice showing signs of stress and about their oral manifestations in the form of recurrent aphthous ulcer, oral lichen planus, and temporomandibular disorders, xerostomia, burning mouth syndrome, bruxism, periodontal disease, dental caries. Identification of such an emotional or psychological disorder stands to benefit both the patient and the practitioner. Consequently, psychological management should be considered when treating patients with any of these psychosomatic disorders. This review literature discussed common oral manifestation of stress.

Keywords: Stress, psychosomatic, anxiety, depression

Introduction

Anxiety and depression are among the psychological disorders that are most prevalent. Such diseases can cause physical and physiological changes in the body, no exception being the oral cavity. Oral diseases involving psychosomatic etiology have been known in medicine for a long time but until now these psychosomatic etiology have not been verified. Since oral mucosa is highly sensitive to emotional factors such as stress, depression and anxiety; oral diseases may occur as a direct emotional experience or as an indirect consequence of psychological changes.1,2 Stress is characterized as an emotional, physically or mentally reaction to the situation that trigger physical or mental stress.3 Oral disease including psychosomatic etiology has long been recognized in medicine and behavioural or emotional factors can serve as a risk factor that can affect oromucosal disease initiation and progression.4,5 Some of the common oral manifestation are given below.

Recurrent Aphthous ulcer

The function of stress that causes in episodes of Recurrent Aphthous Stomatitis (RAS) is still unknown. Acute psychological conditions are typically believed to play a role in the production of repeated aphthous ulcers. Stress may also cause bite of the cheek or lip, or exacerbate actions which cause damage to the oral mucosa. Stressful conditions are expected to induce a temporary rise in salivary cortisol and immune regulation activities in inflammation through raising leukocyte quantities and activity.6 The lack of a clear correlation between stress level and frequency of RAS episodes indicates that psychological stress can serve as a cause or catalyst instead of an etiological factor in susceptible patients with RAS.7

Burning mouth syndrome (BMS)

Depression, anxiety, personal oral dryness, age, prescription, taste disturbances, intake of L-thyroxines, disease and enhanced BMS-associated salivary flow rate, according to study.8 Burning mouth syndrome is related to burning tongue, lips and other mucosal surfaces. Post-menopausal symptoms were assessed significantly by burning throughout the patient’s mouth. Sleep disturbances occurred frequent in patients suffering from burning mouth syndrome. Treatment can be given after finding specific etiological factors for every individual patient including symptomatic relief and management of any related behavioural or psychological disorders.9
Lichen planus (OLP)

Research on the etiopathogenesis of many somatic diseases indicate control of several factors that have additive action, including psychological and social ones. Depending on this, lichen planus is regarded as a psychosomatic disorder, the etiopathogenesis of which is not yet completely understood. The mental and emotional profile of OLP patients was evaluated and the incidence of stress and anxiety was often correlated with an exacerbation of oral lichen planus lesions.\(^5\) It was therefore, also suggested that OLP can be understood as a psychosomatic condition. Study reported a significant correlation between stress and OLP. In addition, the study evaluated a group of patients with anxiety and found a statistically significant higher prevalence of oral lichen planus.\(^10,11,12\)

Xerostomia

Dry mouth syndrome, also known as xerostomia, is an unexplained saliva decrease and may be common in patients with psychological disabilities. Mouth-dryness influences the quality of life.\(^3\) For young adults, the factors are typically associated with stress, anxiety, depression and deficits for nutrition. Consumption of alcohol including the use of illicit drugs can both be significant contributors to dry mouth etiopathogenesis.\(^13,14\) The frequency of reduced secretions of the salivary gland differs in the general population.\(^8\) Overall, xerostomia is more prevalent in females than in males.\(^15\) Secretion of saliva may be influenced by numerous factors like stress, anxiety and depression, age, prior care or cancer radiotherapy, medicines and other factors.\(^16-20\)

Periodontal disease

stress can also have an adverse impact on periodontal tissues. Gum issues are more common in individuals with mild stress.\(^21\) Among other research it was found that along with behavioural and physiological processes stress and depression can be associated with periodontal destruction. In addition, avoidance of dental hygiene during times of stress and depression was related to the loss of periodontal tissues attachment and loss of teeth.\(^22-24\) During World War I, necrotising ulcerative gingivitis has been classically seen from military personnel, possibly due to several risk factors including poor oral hygiene, extreme psychological stress and malnutrition.\(^25\)

Dental caries

Weakens the immune system and reduces stress tolerance to cariogenic bacteria\(^26\) by serum increase, salivary catecholamines and also corticosteroids. The body’s cortisol level is increased throughout stress trying to produce acid that could be determined by litmus testing on the tongue creating a suitable bacterial environment. One research found that children either with or without dental caries differs greatly significantly in urinary catecholamine mean values.\(^27,2\) Reducing salivary secretion resulting in decreased removal of cariogenic bacteria-Subjective oral dryness with unstimulated salivary flow have been significantly correlated with psychological stress \(^28,29,3.\) By serious psychological eating habits that contribute to numerous snacks and more dietary intakes of sugar.\(^4\) By impaired self-care practices (oral hygiene) resulting in poor oral hygiene providing a favourable atmosphere for bacteria.\(^30,31\)

Bruxism

The psychological function is associated with depression, anxiety and mental stress, that perform an important role in the occurrence and perpetuation of bruxism, its severity and frequency. Study found a relationship with anxiety and with signs of depression and symptoms in bruxers. Depressed, nervous, and emotionally stressed individuals are often known to be more predisposed to develop bruxism, particularly during sleep, as a reaction to everyday emotional stress release.\(^32-36\)

Temperomandibular disorder (TMD)

The actual cause of TMD is unclear but is considered multifactorial.\(^8\) Various TMD etiological factors reported in medical literature include psychological factors such as personality traits and behaviour, occlusal differences, inadequate dental care, joint laxity, chronic joint micro damage, overloading / overuse of joint structures, and parafunctional habit. The psychological, physiological, social and emotional problems are also taken into count. Psychosocial factors are most often associated with TMD patients, among various etiological factors of TMD. TMD patients encountered stressful living circumstances before TMD symptoms developed.\(^37-39\)
**Myofascial Pain Disorder**

Schwartz is the first one to include the patient’s psychological structure as a predisposing factor for the condition of myofascial pain disorder (MPDS). According to him, stress was a leading cause of clenching and grinding activities leading to spasms of mastication muscles. Myofascial pain, according to Travell and Simons, stems from trigger points, which are isolated focal hyperirritable spots throughout the taut bands including its skeletal muscles. A research found that long-term musculoskeletal pain conditions affect about 10 per cent of the United States of America’s population. One such musculoskeletal disorder is syndrome of myofascial pain. As Simon and Backstrom have described, stress is characterized as the reaction of the human body to something like a demand imposed upon it, which would lead to negative effect on the body such as MPDS.40-43

**Conclusion**

Patients that shows sign of stress and their oral manifestations in the form of xerostomia, dental caries, periodontal problem, BMS, RAS, OLP, TMD and MPDS frequently come across through their regular practice dentists. Identification of such emotional and psychological illness helps the patient as well as the clinician. Consequently, psychological treatment should be addressed when treating patients with such psychosomatic disorders. Dentists often meet patients with psychosomatic disorders in their everyday practice, patients with emotional disorders often experience oral signs and knowledge of these emotional disorders helps both the patient and the clinician.

**Ethical Clearance**- Not applicable

**Source of Funding**- Nil

**Conflict of Interest**- Nil

**References**


Parental Perception on the Effect of Various Factors in a Dental Clinic Affecting Child’s Behaviour

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Abstract

Background: Societal, professional views and parenting styles have evolved tremendously over the years. Thus, it is important to understand the parental perceptions on the various factors influencing child behaviour in the dental clinic.

Aim: To record and evaluate the parent’s views regarding the various factors that influence child behaviour in a dental clinic.

Materials and methods: A cross sectional study was conducted among 164 parents who were asked to complete a close-ended questionnaire divided into 4 categories- dentist, dental clinical settings, dental treatments and child related factors. This data was analyzed using descriptive statistics.

Results: According to the study results, the parents perceived that the dental treatments and dental settings played a significant role (p< 0.05) that affected the child’s behaviour in a dental clinic. There was no significant differences between the “yes” and “no” responses for the dentist and child related factors.

Conclusion: The parental attitudes and styles are constantly changing as the society evolves. Thus it is important to understand and reassess their beliefs and update our way of managing the dental setup and treatments regularly.

Keywords: child, behaviour, dental care, parent, perception

Introduction

Dental fear prevails to be a major source of problem while managing and treating children in the dental setup. While adult patients tend to develop their own anxiety coping strategies throughout the years, this task often becomes the responsibility of the dentist in pediatric dentistry. The origin of dental fear and uncooperative behaviour of children and proper understanding of it may aid in helping the pediatric dentists to plan and adopt appropriate behaviour management strategies.

Dental fear and behaviour have a multi-factorial origin broadly divided into personal characteristics, environmental factors, or situational factors. Dentists recommend various behaviour guidance methods based upon the child’s health, special health care needs, dental needs, type of treatment required, consequences of no treatment, emotional and intellectual development, parents’ preferences, dentist’s preferences and skills.

The impact of dental atmosphere on child is a major aspect. Every child is considered unique and a major aspect of behaviour management deals with understanding parental perceptions and determining factors that influence child’s behaviour. Over the years, there has been a changing trend in the parenting styles, and societal and professional perceptions. Dentistry always aims to place emphasis on the human aspect of
Thus, it is important to understand the parental perceptions which will present an opportunity to work together and select the best method to provide a safe, effective and comfortable dental visit. Studies related to environmental factors are few and most of the studies were conducted in European and South-East Asian regions. Environmental and situational factors have been previously studied as potential causes of dental fear and behavioural problems but such results have been found to be inconsistent. Hence, the aim of this study was to understand parents’ perception regarding the factors that influence child’s behaviour in a dental setup in Chennai, India.

**Materials and Methods**

The present study was a cross-sectional study conducted in the Department of Paediatric Dentistry, Saveetha Dental College and Hospitals. The study design was reviewed and approved by the Institutional Review Board (SDC/PEDO-1704/19/014).

**Participant selection:**

A total of 170 parents who visited the Department of Pediatric Dentistry for their child’s dental treatment were included. Amongst that 164 parents agreed to participate in the study. The parents of children who required special needs or have associated systemic conditions were excluded. Thus, parents of children with normal developmental milestones and with at least one prior dental visit were included in the study.

A validated questionnaire was provided to the parents and asked to complete within treatment hours. The questionnaire comprised of 16 close ended questions divided into 4 categories: dentist, dental setting, dental treatment and child related factors.

**Statistical Analysis**

Based on the information collected, responses were recorded, processed and a descriptive analysis was followed.

**Results**

The results of 164 participants were tabulated and analyzed accordingly. The majority of the parents who brought their child for dental treatment belonged to the age range of 31-40 years (52%) and the least belonged to 41-50 years (17%) with a distribution of 43% males and 57% females (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>51</td>
<td>31</td>
</tr>
<tr>
<td>31-40</td>
<td>85</td>
<td>52</td>
</tr>
<tr>
<td>41-50</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>70</td>
<td>43</td>
</tr>
<tr>
<td>Female</td>
<td>94</td>
<td>57</td>
</tr>
</tbody>
</table>

The major reason for bringing the child for dental visit (Table 2) was pain (40%) followed by decay (24%), routine check-up (15%), trauma (11%) and other reasons (10%). According to responses recorded from parents in terms of yes or no (Table 3), the probabilities of the t-test for the four parameters under evaluation were dentist (p=0.0989), dental settings (p=0.0187), dental treatment (p=0.00018), and child related factors (p=0.17809).

<table>
<thead>
<tr>
<th>Reason</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decay</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>Pain</td>
<td>65</td>
<td>40</td>
</tr>
<tr>
<td>Trauma</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Routine check-up</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3: Parent’s perception on various factors affecting the behaviour of their child in the clinic

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>%</th>
<th>NO</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DENTIST</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dentist attitude(friendly/stern)</td>
<td>115</td>
<td>70</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td>Dentist attire(with or without lab coat)</td>
<td>99</td>
<td>60</td>
<td>65</td>
<td>40</td>
</tr>
<tr>
<td>Gender</td>
<td>92</td>
<td>56</td>
<td>72</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>306</td>
<td>62</td>
<td>186</td>
<td>38</td>
</tr>
<tr>
<td><strong>DENTAL SETTINGS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleasantness of dental setup (colour, decoration, etc.)</td>
<td>117</td>
<td>71</td>
<td>47</td>
<td>29</td>
</tr>
<tr>
<td>Friendliness of staff</td>
<td>115</td>
<td>70</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>232</td>
<td>70.5</td>
<td>96</td>
<td>29.5</td>
</tr>
<tr>
<td><strong>DENTAL TREATMENT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of procedure</td>
<td>120</td>
<td>73</td>
<td>44</td>
<td>27</td>
</tr>
<tr>
<td>Type of procedure (filling, scaling, extraction, etc.)</td>
<td>122</td>
<td>74</td>
<td>42</td>
<td>25</td>
</tr>
<tr>
<td>Dental appointment timing(morning/afternoon)</td>
<td>99</td>
<td>60</td>
<td>72</td>
<td>40</td>
</tr>
<tr>
<td>Sound and noise of instrument</td>
<td>130</td>
<td>79</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>Absence/Presence of parent’s during procedure</td>
<td>112</td>
<td>63</td>
<td>52</td>
<td>31</td>
</tr>
<tr>
<td>Behaviour of other children undergoing treatment</td>
<td>99</td>
<td>60</td>
<td>65</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>682</td>
<td>69</td>
<td>309</td>
<td>31</td>
</tr>
<tr>
<td><strong>CHILD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of the child</td>
<td>84</td>
<td>51</td>
<td>80</td>
<td>49</td>
</tr>
<tr>
<td>Gender of the child</td>
<td>79</td>
<td>48</td>
<td>85</td>
<td>52</td>
</tr>
<tr>
<td>Previous dental experience of the child</td>
<td>96</td>
<td>59</td>
<td>68</td>
<td>41</td>
</tr>
<tr>
<td>Diet consumed by the child</td>
<td>61</td>
<td>37</td>
<td>103</td>
<td>63</td>
</tr>
<tr>
<td>Previous dental experience of peers/ and siblings</td>
<td>82</td>
<td>50</td>
<td>82</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>402</td>
<td>49</td>
<td>418</td>
<td>51</td>
</tr>
</tbody>
</table>

According to the results, the parent’s perception in respect to two parameters such as dentists and child factors, there was no significant difference noted between the two responses [Yes or No] (p> 0.05), whereas there was a significant difference noted for the factors: dental settings and dental treatment (p<0.05).
Discussion

Getting to understand and learn about parents’ perceptions regarding various factors in dental clinic and thereby making the parent understand about the influence of various factors in dental clinic play a major role in establishing a positive dental outcome [12].

As per the study results, 62% of parents agreed that the attitude, attire and gender of the dentist affect the child’s behaviour in dental clinic. Amongst that, dentist’s attitude (70%) and dentist attire (60%) were believed to play a role in child behaviour. There was no major difference in the results when the gender of the dentist was taken into criteria. Thus, to gain the trust of the children and parent, the dentist’s attitude, body language, and communication skills play a crucial role in creating a positive dental experience for the child and parent [13].

Over the years, there has been a concern that the white coat and professional clothing worn by the dentist can increase anxiety in the children. The protective mask may also act as a stressor for the child and thus it is recommended to introduce the child to the dental environment without the mask [14]. It was found that 58% of parents preferred non-white coat attires and formal attires among dentists [15, 16]. In a study conducted in India, it was noted that children preferred their dentists to wear dental scrubs and white coat but were highly anxious on seeing their dentist with any protective wear [17].

American Academy of Pediatric Dentistry (AAPD) describes that the orientation of dental environment and atmosphere play an important role influencing the child’s behaviour in dental clinic [13]. According to the present study, friendliness of staff and pleasantness of the dental set up such as colour, decoration etc. affects the child’s behaviour according to 70% and 71% of parents respectively. Thus a cumulative of 70.5% parents agreed that the dental settings in general played a crucial role in child’s behaviour in dental clinic. The results are in accordance with another study which exhibited the effects of the atmosphere and dental settings on the child’s behaviour and emotions [18]. The children exhibited positive emotions towards bright colours (e.g., pink, blue) and negative emotions towards dark colours (e.g. black, brown) [19]. Thus the use of child friendly colours like blue and yellow enhances a positive dental attitude in the child’s mind [20].

Dental healthcare environment is getting more competitive everyday and patients are more demanding than before [21]. The results show that the dentists should pay attention to the dental settings in addition to focussing on the treatment which in turn will motivate the children to undergo the dental procedure in a well behaved manner [5].

According to our study results, 69% of parents agreed that the dental treatment has an effect on the child’s behaviour. Amongst that, 73% agreed that the treatment duration affects the child’s behaviour, which is similar to previous studies [5, 22]. The dental instrument’s sound and noise influence the child’s behaviour according to 79% of parents and the type of dental procedure was believed to have some effect on the child according to 74% parents. In a similar study, 77% of parents agreed that the type of procedures, such as drilling/ restoration and the sight of instruments cause fear and anxiety in the child; however, it is known that procedures like injection may produce a high level of anxiety [23].

63% of parents agreed that the presence of parents during treatment might influence the behaviour of the child. However, a study stated that the presence/absence of parents did not have any effect on the child’s behaviour [24]. When the timing of the dental appointment was taken into criteria, 56% of the parents in our study felt that early morning or afternoon dental appointments had some impact on the child’s behaviour. Early morning appointments are preferable for young children as they are more rested and thus cooperative. The children may become tired or cranky from missing nap during afternoons or later periods of the day [25]. However according to a study by Lechner, timing of the day of the dental appointment had no significant effect on child’s behaviour [26]. Thus, it can be stated that time scheduling for dental appointment should be more dependent on convenience than possible behaviour effect based scheduling [27].

49% of parents in our study stated that the child related factors such as age, gender, previous dental experience and diet of a child including the previous dental experience of the child’s sibling had an influence on the child’s behaviour. A longitudinal behavioural
study stated that the child undergoing dental care was directly influenced by the psychomotor development of the child [28]. 59% of parents agreed that previous dental experience of the child might have influenced the child’s cooperation towards the dental treatment. It is said that a previous traumatic experience in a dental clinic or during hospitalization for any other reason can also provoke anxiety and fear in the child [29, 30].

Previous dental experience of the child’s sibling and the gender of the child were considered to impact the child’s behaviour according to 50% and 48% of the parents in this study. One study stated that girls are more willing to visit the dentist and cooperate compared to boys of the same age [23]. The diet consumed by the child is believed to affect the child’s behaviour according to 37% of parents in the current study. Diet is proven to affect the cognitive ability and behaviour of children and adolescents [31].

This study is the first of its kind carried out in India for the evaluation of parental perception about various factors affecting the child’s behaviour in a dental environment. One possible limitation of this study might be the small sample size. Thus, further research in a prospective intervention based method is required to ascertain whether understanding of parental perceptions can serve to be an effective tool in the prevention of child’s behavioural problems; and also study the changing trends in various countries.

Conclusion

Dental treatment and dental settings play a key role affecting the child’s behaviour according to the perception of the parents in our study. The success of any paediatric dental practice not only depends on the skills of the dentist but also depends on the patients and their parents. Thus gaining a positive dental experience from both the child and the parent is necessary. This relies on the understanding and constant updating of the changing parental styles and attitudes of the society.

Conflicts of Interest: None.

Source of Funding: Self.

Ethical Clearance: Taken from Saveetha Institute of Medical and Technical Sciences.

References

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Effectiveness of Video Assisted Teaching Programme on Knowledge and Expressed Practices Regarding the First Aid Management of Epilepsy among Patient’s Family Members in Selected Hospitals of Ambala, Haryana

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Abstract

The epilepsy is a symptom complex of several disorders of brain function characterized by recurring seizures. Practically epilepsy is not a disease but a symptom that may lead to further mental diseases. It includes loss of consciousness, excess movement or loss of muscle tone or movement, disturbances of behaviour, mood, sensation, and perception. Only the patient experiences the Epileptic fits or seizures causing a “sensation” within him/her. Most important thing in the epilepsy first aid management we have to stay calm; most seizures last less than a few minutes. Help the person having the seizure from getting hurt by moving any nearby objects out of the way, as necessary. The research includes Quantitative approach and quasi experimental non-equivalent control group pretest-posttest design. Total 60 patient’s family members (30 in experimental group and 30 in comparison group) were recruited. Conveniently 1 hospital were selected and then the sample was chosen by convenience sampling technique. Tools in the study were selected variables, structured knowledge questionnaire and structured expressed practices scale. The mean post-test knowledge score of experimental group was higher (14.67) than the mean post test score of comparison group (9.77) which was found to be statistically significant (‘t’ = 2.21, p = 0.00**) at the 0.05 level of significance. The mean post-test expressed practices score of the experimental group was higher (91.87) than the mean post-test expressed practices score of comparison group (74.50), which was found to be statistically significant (‘t’ = 14.46, p =0.00**) at the 0.05 level of significance. No significant association was found among knowledge scores and expressed practices scores regarding first aid management of epilepsy among patient’s family members with selected variable. Video assisted teaching was effective to improve the knowledge and expressed practices regarding First aid management Epilepsy among patient’s family members as there was a significant difference between experimental and comparison group.

Key words: Video assisted teaching, Knowledge, Expressed Practices, Patient’s family members, First Aid, Epilepsy

Introduction

Chronic non-communicable illnesses are a major health care problem in evolving countries and neuropsychiatry disorders reason for more than a quarter of the global burden of disease. Mental sicknesses are major contributor to the universal drain of disease. From this, epilepsy is one of the most common and serious brain disorders in the world. It affects at least 50 million people in the world including Asian countries. The pervasiveness of active epilepsy in developing countries range from 5 to 10 per 1,000 people. The worldwide prevalence rate of epilepsy varies from 2.8 to 19.5 per
1,000 of the general population and is more prevalent among children. The estimated prevalence of epilepsy is 8–13 per 1,000 people.1

“Epilamabavian” means “to seize or to take hold of”. The term “epilepsy” is derived from such a Greek word. To the Greeks, epilepsy was a sacred disease; it was a disease of the brain. Later, it become known as “the falling sickness diseases condition”. Regardless of the insight gained into epilepsy, lack of knowledge, stigma and fear are still associated with this problem. People think epilepsy is a curse from god1

The epilepsy is a symptom complex of several disorders of brain function characterized by recurring seizures. Practically epilepsy is not a disease but a symptom that may lead to further mental diseases. It includes loss of consciousness, excess movement or loss of muscle tone or movement, disturbances of behaviour, mood, sensation, and perception.2 Only the patient experiences the Epileptic fits or seizures causing a “sensation” within him. Perception of a strange light, unpleasant thought, visual changes, unpleasant smell and auditory changes are some of those sensations. These sensations are subjective feelings commonly known as ‘Aura’. Strong emotions, intense exercise, music or flashing lights can be the cause for activating the seizure’s in few people.2

Inadequate knowledge regarding the treatment routine and first aid management of the epileptic fits has caused various injuries (loss of teeth, injuries to the head, fracture and dislocation) during the epileptic fits. Various practices have also been documented such as placing a key in the hand of the patient, administered liquid orally, applying pressure to restrain the body, putting some objects into the mouth to force the teeth open.

**Material and Method**

The is a quasi-experimental study was conducted during the period from October 2019 to January 2020 in Haryana, India. Total 60 patient’s were selected from Maharishi Markandeshwar Institute of medical Sciences and Research and Hospital OPD (Psychiatric department 30 in experimental and 30 in comparison group) by using convenience sampling technique. Video assisted teaching programme was provided to experimental group.

**Inclusion criteria**

Study included patient’s family members who were

- Available at the time of data collection
- Willing to participate in the study
- Who can understand Hindi

**Exclusion criteria**

The exclusion criteria excluded teachers who were:

- Not available at the time of data collection

**Phase I:** The investigator visited Psychiatric OPD all days from Monday to Saturday. A structured knowledge questionnaire and expressed practices scale was used for the data collection after obtaining informed written consent. The study participants were given Video Assisted Teaching Programme on first aid management of epilepsy. In this VATP, all the details like epilepsy, causes of epilepsy manifestations and role of family members in first aid management thought provoking video to educate them.

**Phase II:** 7 days after intervention period, the post test were conducted when family members comes along with patient’s again. Pre and Post test results were analysed using SPSS 20.

**Study tool and study variables:** Part A- Selected variables Details age, gender, qualification, marital status, treatment, religion, stay with patient, previous knowledge on first aid management for epilepsy. Part B- Structured knowledge questionnaire the investigator asses the knowledge by using Structured knowledge questionnaire. Part C Structured expressed practices scale the investigator asses the knowledge by using Structured expressed practices scale.

**Results**

**Distribution of the data based on** mean, mean difference, standard deviations of difference, standard error of mean difference and ‘t’ value of knowledge score of patient’s family members regarding First Aid Management of epilepsy before administration of VATP in experimental and comparison group. Table no. 1=
The mean knowledge score (9.40) of experimental group was lower than the mean knowledge score of (9.67) of comparison group with the mean difference of 0.26. The computed ‘t’ value (0.54) was found to be statistically non-significant at 0.05 level of significance which inferred that both the groups were homogenous in terms of knowledge at the baseline. Table no= 2 expressed practices before administration of VATP regarding First Aid Management of epilepsy in experimental and comparison group. The mean Expressed practice score (68.57) of experimental group was higher than the mean Expressed practice score (67.13) of comparison group with the mean difference of (1.43) the computed ‘t’ value (1.30) and (p=0.19) was found to be statistically non-significant at the level of 0.05 level of significance which inferred that both the groups were homogenous in terms of expressed practice at the baseline. Table no=3 in experimental group, there was statistically significant weak positive correlation between knowledge and expressed practice and was statistically significant at 0.05 level of significance, but in comparison group, there was no statistically significant correlation between knowledge and expressed practice.

Table 1: Mean, mean difference, standard deviation of difference, Standard Error of Mean Difference and ‘t’ value of Knowledge among Patient’s family members after VATP in experimental and comparison group. (N=60)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD</th>
<th>MD</th>
<th>SEMD</th>
<th>‘t’ value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group(n=30)</td>
<td>14.67±1.51</td>
<td>4.90</td>
<td>0.40</td>
<td>2.21</td>
<td>58</td>
<td>0.00*</td>
</tr>
<tr>
<td>Comparison group(n=30)</td>
<td>9.77±1.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean, mean difference, standard deviation of difference, Standard Error of Mean Difference and ‘t’ value of Expressed practices among Patient’s family members after VATP in experimental and comparison group. (N=60)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD</th>
<th>MD</th>
<th>SEMD</th>
<th>‘t’ value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group(n=30)</td>
<td>91.87±3.64</td>
<td>17.36</td>
<td>1.20</td>
<td>14.46</td>
<td>58</td>
<td>0.00*</td>
</tr>
<tr>
<td>Comparison group(n=30)</td>
<td>74.50±5.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Correlation between knowledge and expressed practices regarding first aid management of epilepsy among patient’s family members in both experimental and comparison group. (N=60)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Expressed practices</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental (n=30)</td>
<td>Knowledge</td>
<td>Pre 0.18(0.92NS)</td>
<td>XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post XX</td>
<td>0.18(0.03*)</td>
</tr>
<tr>
<td>Comparison (n=30)</td>
<td></td>
<td>Pre 0.025(0.277NS)</td>
<td>XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post XX</td>
<td>0.29(0.11NS)</td>
</tr>
</tbody>
</table>

Discussion

The present study aimed to evaluate the effectiveness of video assisted teaching on knowledge and expressed practices regarding first aid management of epilepsy among patient’s family members in selected Hospital of Ambala, Haryana. The total number of patient’s family members is 60. In present study, half of the family members (33%) belong to age group of less than 40 years. These findings are consistent with the study conducted by Howard Ring, James Howlett, Mark Pennington (2018) where they found that half aged 18–65 years with an ID and epilepsy under the care of a community ID team and had had at least one seizure in the 6 months before the trial.

Conclusion

The findings of the study results show that Video Assisted Teaching Programme (VATP) effective in increasing the knowledge and expressed practices among patient’s family members as there was a significant difference between the experimental and comparison group.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: The study was approved by the Ethics committee of the MMDU institute.

References

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Variation in the Shapes of Coronoid Process of Dry Human Mandible of Gujarat Region

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Abstract

Introduction: Coronoid process is single the bony processes of the ramus of the mandible. It is a thin, triangular eminence, which is flattened from side to side. It differ in shape and size. The importance of frequency in the shapes of Coronoid process is important for maxillofacial surgeons as a graft material as well as for anthropologists for the detection of races.

Aim: To determine the different shape of Coronoid process of mandible in both sides.

Materials and Method: The Present study was conducted on 50 mandibles from the department of Anatomy, Baroda Medical College, Baroda. Variations in the shapes were observed, and photographs were taken.

Results: Triangular shape of Coronoid process was most prominent as compare to hook and round shape. Triangular shaped Coronoid was seen in 70% shaped (Type: I) followed by Rounded shaped 26%, Hook shaped was least 4% respectively.

Conclusion: This study will be helpful for maxillofacial surgeons, anatomists, forensic researchers, anthropologists.

Key Words: Mandible, Coronoid process, Triangular, Rounded, Hook.

Introduction

The mandible is the bone of the jaw bone. It is the largest, strongest, and lowest bone of the face and bears the lower teeth. The mandible is horseshoe-shaped and consists of Three parts: a horizontally-oriented body and two vertically oriented rami.

Body: The body of the mandible is U-shaped and it presents:

1. Two surfaces
   (a) External surface.
   (b) Internal surface.

2. Two borders
   (a) Superior border of the body is called alveolar process.
   (b) Inferior border or base of the mandible presents a small depression digastric fossa on either side near the median plane.

   It gives attachment to the anterior belly of the digastric.

Characteristic on the external and internal surfaces of the body:

The outer surface of the body of the mandible presents the following features:

Symphysis menti: It is a faint median ridge on the external surface of the body.

It marks the line of fusion of the two halves of the mandible at the age of 2 years.
The symphysis menti expands below into a triangular elevation termed mental protuberance.

It forms the point of chin, the base of which is limited on each side by the mental tubercle.

The inner aspect of symphysis menti possesses four tubercles called genial tubercles arranged into two pairs: upper and lower.

The upper pair provides attachment to genioglossus muscles and lower pair to geniohyoid muscles.

Mental foramen: It lies below the interval between the premolar teeth and provides passage to mental nerve and vessels.

Oblique line: It is the continuation of the anterior border of the ramus.

It runs downwards and forwards to with regards to the mental tubercle.

Incisive fossa: It is a shallow depression just below the incisor teeth.

The internal surface of the body in each half of the mandible presents the following features

I) Mylohyoid line is a prominent oblique ridge that runs obliquely downwards and forwards from behind the 3rd molar tooth to the symphysis menti below the genial tubercles.

II) Mylohyoid groove lies below the posterior end of the mylohyoid line. Mylohyoid nerve and vessels run in this groove.

III) Sublingual fossa is a shallow area above the anterior part of the mylohyoid line and lodges sublingual gland.

IV) Submandibular fossa is a slightly hollowed out area below the posterior part of the mylohyoid line and lodges submandibular gland.

Ramus of the Mandible

It is more or less a quadrilateral vertical plate of bone that projects upwards from the posterior part of the body.

The ramus presents the following features:

Two surfaces

(a) Lateral surface.

(b) Medial surface.

Four borders

(a) Anterior.

(b) Superior

(c) Inferior.

(d) Posterior.

3. Two processes

(a) Condylar process, a strong upward projection from postero superior part.

Its upper end is expanded to form head. Neck is the constricted part below head

and presents a depression on its anterior surface called pterygoid fossa.

(b) Coronoid process, a flattened (side to side) triangular protrusion from anterosuperior part.(2,13)

Coronoid process derived from the Greek word “korone” meaning “like a crown.

The Coronoid process of mandible is a thin, triangular process projects upward and slightly forward.

This process gives attachment to two important muscles of mastication Temporalis muscle attached to apex whole of the medial surface and anterior part of lateral surface.

Rest of the lateral surface gives attachment to masseter.

It varies in shape and size.

The Knowledge of variation in the shapes of Coronoid process is important for maxillofacial surgeons as a graft material as well as for anthropologists for the detection of races.(3,7)
Aims and Objectives

Aims: To determine the different shape of Coronoid process of mandible in both sides.

Objectives

Depending upon the observation was categories into 3 types.

I Type 1: Triangular: the tip of coronoid process straight upward.

II Type 2: Hook shaped in which the tip of coronoid process was pointing backward.

III Type 3: Rounded: tip is rounded.

Materials and Method

The cross sectional study was performed in Department of Anatomy in Baroda medical college, Vadodara, Gujrat.

A total of 100 dry mandible (50 right side and 50 left side) bones were studied from teaching collection of the Anatomy department. The bones belonged to mature specimens but the exact ages and gender of the specimens were not known. All the mandible selected were dry, complete and showed normal anatomical features and bones showing pathology were excluded from study.

All measurement was carried out by the same instrument.

The data were then entered and analysed using the Microsoft Excel 2010.

The following parameters of the shape of Coronoid process mandible were observed.

SHAPE OF THE CORONOID PROCESS:
Different shape of Coronoid process of mandible in both sides.(6,8)

OBSERVATION

Figure I showing triangular shaped coronoid process which is more observed in current study.(6,7,13)
**TYPE:II ROUNDED SHAPE**. Figure II showing Rounded shaped coronoid process which is less observed in current study.

**TYPE :III HOOK SHAPE**

Figure III showing Hooked shaped coronoid process which is very few observed in current study.
### Results

Frequency of various shape of coronoid process in total side with percentage. (6, 7, 13)

<table>
<thead>
<tr>
<th>Type</th>
<th>Shape</th>
<th>Total</th>
<th>Percentage</th>
<th>Bi lateral</th>
<th>Unilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bi lateral</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Percentage</td>
<td>Percentage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right</td>
<td>left</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Percentage</td>
</tr>
<tr>
<td>I</td>
<td>Triangular</td>
<td>70</td>
<td>70%</td>
<td>40</td>
<td>57%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>10</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>43%</td>
</tr>
<tr>
<td>II</td>
<td>Rounded</td>
<td>26</td>
<td>26%</td>
<td>20</td>
<td>76%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>24%</td>
</tr>
<tr>
<td>II</td>
<td>Hook</td>
<td>4</td>
<td>4%</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Frequency of various shape of coronoid process**

**Total**

In this figure, the graph shows the frequency of various types of coronoid process in percentage.
Discussion

Differentiation of Vary Studies of Coronoid Process of Mandible.

<table>
<thead>
<tr>
<th>Frequency of shape of Types of coronoid Process</th>
<th>Triangular Shaped Percentage</th>
<th>Rounded Shaped Percentage</th>
<th>Hook Shaped Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issac B (2001)11</td>
<td>49</td>
<td>27.4</td>
<td>23.6</td>
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<td>sNirmale et al (2012)14</td>
<td>65</td>
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<td>7</td>
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<td>Smita Tapas (2014)15</td>
<td>60</td>
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<td>Vikas Desai (2014)16</td>
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<td>8</td>
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<td>Sheela Kadam (2015)17</td>
<td>64.97</td>
<td>21.02</td>
<td>14.01</td>
</tr>
<tr>
<td>Priyank Bhabhor (2015)18</td>
<td>29.65</td>
<td>45</td>
<td>25.35</td>
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<td>quadric(2016)19</td>
<td>67</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>CURRENT STUDY</td>
<td>70</td>
<td>26</td>
<td>4</td>
</tr>
</tbody>
</table>

Tanveer A(2011)13, Vikas Desai (2014)16, A Quadri that observed variation in shape of coronoid process triangular shaped frequency near by to what observed in current study.(6,7)


Conclusion

Current study, out of 50 mandibles triangular shaped coronoid process was establish to be majority wide spread keep to rounded shaped next hook shaped.

i) Awareness of frequency of shape of coronoid process helpful for the maxillofacial surgeons as it can be used as an excellent graft for reconstructive surgeries .(3)

ii) frequency shape of coronoid process caused by unilateral chewing habits, muscular pull on the process and hormonal factors.(3)

iii) Knowledge of Variation In Shape is useful Dental Surgeons In Oral & Maxillofacial Surgeries.(7)
IV) Coronoid process can be easily garner as a donor bone.

V) The coronoid process appear to be suitable for paranasal augmentation.

application is also use because its size and morphology fits into the paranasal region, with the advantages of biocompatibility, availability, and reduced surgery time for harvesting.

Ethical Clearance: Taken Sumandeep Vidyapeeth.

Source of Funding: Self

Conflict of Interest: Nill

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Development of Physical and Mental Abilities in Fulfilling Children’s Rights in the Program of ‘Kampung Anak Negeri’ in Surabaya

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Abstract
As an obligation to protect the children, both physically and mentally, every child must be provided in accordance with their welfare by providing guarantees for the fulfillment of children’s rights. The Indonesian government’s concern for the protection and fulfillment of children’s rights has actually existed since the enactment of Law Number 4 of 1979 concerning Child Welfare. However, until the promulgation of the Child Protection Act to date, welfare and fulfillment of children’s rights are still far from what they should be, mostly for street children and neglected children. The purpose of this research is to study how the efforts to fulfill children’s rights through the program specifically for street children and neglected children. The method used in this research is normative juridical. Based on the results of the study, that the Kampung Anak Negeri Program in Surabaya was made as a social service for the fulfillment of children’s rights in the orphanage system, including meeting the needs of food, clothing, and shelter, as well as mental and spiritual guidance, behavior, children’s talents, and business skills that can improve child welfare after leaving later.

Keywords: physical development, education, mental abilities, children’s rights, Kampung Anak Negeri, Surabaya.

Introduction
The Indonesian state guarantees the protection of the human rights of its citizens, including the rights possessed by children. This is marked by the protection and fulfillment of children’s rights in the 1945 Constitution of Indonesia as well as several other laws and regulations, both national and international. The protection of children’s rights is strengthened by the ratification of the Convention on the Rights of the Child through Presidential Decree Number 36 of 1990 concerning Ratification of the Convention on the Rights of the Child.¹

The principles of protection for children, among others, are regulated in the Convention on the Rights of the Child which has been ratified by the government through Presidential Decree No. 36 of 1990 which sets out general principles for child protection, namely non-discrimination, the best interests of the child, survival, and child development, and respect for children’s participation. This principle is also accommodated in the provisions of Law Number 35 of 2014 concerning Amendments to Law Number 23 of 2002 concerning Child Protection. This is done so that the principles of protecting children can be applied in Indonesia. The Constitution stated that the poor and neglected children are interrupted by the State. These neglected children also have rights as a whole child who must also be given protection and guarantee for the fulfillment of their children’s rights.

The Indonesian government’s concern for the protection and fulfillment of children’s rights has actually existed since the enactment of Law Number 4 of 1979 concerning Child Welfare. However, until the issuance of the Child Protection Law to date, the welfare and fulfillment of children’s rights, especially street children
and neglected children, are still far from being expected. Abandoned children according to Article 1 point 6 of Law Number 35 of 2014 concerning Child Protection are children whose needs are not properly fulfilled, whether physically, mentally, spiritually, or socially. Street children are an example of neglected children. Many parties underestimate street children or children with juvenile delinquency problems. However, the Surabaya Government does not have high concern for the future of street children in Surabaya. This is evidenced by the establishment of the program of Kampung Anak Negeri which is under the guidance of the Surabaya Social Service. The program was established with the aim of fostering street children, school dropouts, and children with Social Welfare Problems.

Research Methods

The approach method used in this research is the normative juridical approach method. The normative research method is a study that analyzes law both written in the book, as well as law that is decided by the judge through judicial process. This research uses descriptive analytical research method, which is research that generally aims to describe systematically, factually and accurately on a certain matter, regarding the characteristics, characteristics, or certain factors related to the fulfillment of children’s rights. Data collection techniques that will be used in this study, the authors use literature study. To increase the source of data in this study, interviews were also conducted with the management of the Kampung Anak Negeri Surabaya, thus completing the results of this study.

Children’s Rights in Indonesian Laws and Regulations

The rights of the child based on Article 52 paragraph (2) of Law Number 39 of 1999 concerning Human Rights are human rights and for its interests the rights of the child are recognized and protected by law even when in the womb. Children’s rights apply when the child is in the womb where the child needs protection to live. Then when he is born he needs to maintain his life and wants an increase in the standard of living.

Children have the right to legal protection from all forms of physical or mental violence, neglect, ill-treatment and sexual harassment while they are in the care of their parents or guardians, or other parties who are responsible for caring for the child. In addition, children are entitled to protection from every activity of economic exploitation and any work that endangers them so that they can interfere with their education, physical health, moral, social and mental spiritual life.

Law No. 39 of 1999 concerning Human Rights stated that children have the right to protection by parents, family, society and the state; children with physical and or mental disabilities have the right to receive special care, education, training and assistance at the expense of the state, to ensure their life in accordance with human dignity, increase self-confidence and the ability to participate in the life of society, nation and state; children have the right to worship according to their religion, think and express themselves under the guidance of their parents and/or guardians; children have the right to know who their parents are, to be raised and cared for by their own parents. When the child’s parents are unable to raise and care for their child properly and in accordance with this law, the child may be cared for or adopted as a child by another person in accordance with the provisions of the laws and regulations; children have the right to receive education and teaching in the framework of personal development according to their interests, talents and intelligence level; children have the right to associate with children of the same age, play, have recreation, and be creative according to their interests, talents and level of intelligence for self-development; children have the right to get proper health services and social security, in accordance with their physical and mental and spiritual needs; the child has the right to receive protection from economic exploitation activities and any work that endangers him, so that it can interfere with his education, physical health, moral, social life, and mental spirituality; children have the right to receive protection from activities of sexual exploitation and abuse, kidnapping, child trafficking, and various forms of abuse of narcotics, psychotropic substances and other additives; children have the right not to be subjected to mistreatment, torture, or inhuman punishment. The death penalty or life sentence cannot be imposed on a child offender.

Law No. 35 of 2014 concerning Amendments to Law Number 23 of 2002 concerning Child Protection stated that child protection is all activities to guarantee
and protect children and their rights so that they can live, grow, develop, participate in an optimal manner in accordance with human dignity, and receive protection from violence and discrimination. Children’s rights are part of human rights that must be guaranteed, protected and fulfilled by parents, family, community, government and the state. Children’s rights in Law No. 35 of 2014 stated that children have the right to live, grow, develop and receive protection from violence and discrimination; children have the right to worship according to their religion, think and express themselves according to their level of intelligence and age; children have the right to know their parents, to be raised and cared for by their own parents. If the parents cannot guarantee the child’s growth and development, or the child is neglected, the child has the right to be cared for or adopted as a foster child or adopted child by someone else in accordance with the provisions of the applicable laws and regulations; children have the right to obtain health services and social security in accordance with their physical, mental, spiritual and social needs; children have the right to education and teaching in the context of personal development and intelligence level according to their interests and talents. Especially for children with disabilities are also entitled to receive special education, while for children who have advantages are also entitled to special education; children have the right to rest and take advantage of their spare time, associate with children of the same age, play, have recreation, and be creative according to their interests, talents and intelligence level for self-development; children with disabilities are entitled to rehabilitation, social assistance and maintenance of social welfare; children have the right to receive protection from acts of abuse, torture, or violence.

Development of Physical and Mental Abilities in Surabaya’s Kampung Anak Negeri

Legal products of the laws and regulations governing the protection of children’s rights in Indonesia have provided a comprehensive and adequate aspect in children’s rights. However, in implementation in community life there are still many shortcomings in fulfilling children’s rights, including many cases such as street children, dropped out of school, exploitation of children for the economy, physical and non-physical violence, and sexual violence. However, the fulfillment of children’s rights has not been carried out optimally, especially for street children, school dropouts, and children with Social Welfare Problems. Their presence is increasing in number, causing anxiety for the general public if they are not properly nurtured. Because it is feared that it will have a negative impact on public order and security. Homelessness creates risky conditions for homeless children and adolescents, for example, malnutrition, loss of parental support, affiliation with deviant peers, dropping out of school, and so on. This state of risk has devastating physical, emotional, social and educational impacts on young people.

Based on these conditions, since 2009 the Surabaya Government has established the Kampung Anak Negeri Program as a sustainable and structured program in order to foster and ensure the fulfillment of the rights of street children, school dropouts, and children with social welfare problems in Surabaya. This program was formed as an effort to create and provide a decent life for them because they are an integral part of the state as the nation’s next generation. According to Erni Lutfia, Head of the Technical Implementation Unit of Kampung Anak Negeri, the children here come from various backgrounds. They come from street children, school dropouts, and children with social welfare problems who were caught by the raids on the municipal police. Until now, a total of 35 children live in Kampung Anak Negeri. Their age range is between 7 and 18 years. Education is an essential right to citizen. Article 28 of the Convention on the Rights of the Child (1989), recognizes the right of every child to a free primary education, and encourages the development of secondary education that is accessible and available. This right is essential to all societies in order to promote and achieve of stable and harmonious relations among nations and build the good citizens for the future.

The education provided in Kampung Anak Negeri is in the form of formal and non-formal education. For formal education, Kampung Anak Negeri collaborates with nearby schools. Meanwhile, for children who have dropped out of school or dropped out, they will be included in pursuing packages, so that they can still receive education. On the other hand, non-formal education is in the form of interest and talent training, as well as entrepreneurial skills.
The shelter in Kampung Anak Negeri is somewhat different from a shelter. Children are free to play even outside the shelter area. However, there are still disciplinary boundaries that they must obey, such as going to school, attending interest and talent training, praying and reciting the Koran. So that the method applied can make children who live feel comfortable and have fun. The approach taken by the companions there is also somewhat different from the others, a companion must understand the child’s psychology. Not only that, the figure of the companion there must also be a substitute father or mother for them, so that they still feel affection. Through the programs that have been carried out, some of these children have both regional and national achievements. For example, a child, AM (14) won first place in the 38 kilogram class boxing competition in the East Java Youth Amateur Boxing Championship in 2017. In bicycle racing, a child, MM (7) won third place in the KONI Bike Race Championship in Surabaya. Another child, LAP (16) has won in the same field second place in the Third Series Early Age Bike Race, Chairman of ISSI Central Java. From the martial art branch, MH (14) has won the award for early childhood champion in Surabaya.

While living in Kampung Anak Negeri, the children will be financed for their education until they graduate by the Surabaya Government, and even assist them to find work. In fact, there has been cooperation with five-star hotels in Surabaya, so that they can work there. In addition, they are also taught entrepreneurship, such as being a barista at the Children’s Coffee Shop belonging to the Surabaya Social Service. The method applied in the program has shown success, as many of these children have graduated from school and found jobs. The program received appreciation from the Minister of Women’s Empowerment and Child Protection, and plans to adopt the program to be implemented throughout Indonesia. The program is also one of the supporters of Surabaya being designated as a City Fit for Children in 2019 with the main title.

**Conclusion**

The fulfillment of children’s rights, especially for street children and school dropouts, in the Kampung Anak Negeri in Surabaya has been carried out well. Children are in the phase of growth and development, so they need to get guidance and shade in order to have mental and social maturity. Initially they live and live on the streets and drop out of school, transforming into a better life. They are given shelter and shelter. In addition, they are also provided for their primary needs, educational rights, mental and spiritual guidance. So that children can have the widest opportunity to grow and develop optimally both physically, psychologically and in social life.

**Ethical Clearance:** This research was ethically approved by Faculty of Law, Universitas Diponegoro, Semarang, Indonesia.

**Funding:** This research receives funding of research grant from Faculty of Law, Universitas Diponegoro, Semarang, Indonesia.

**Conflict of Interests:** There are no conflict of interests

**References**


Awareness of Medico Legal Aspects of Clinical Practise and Common Medico Legal Issues among Medical Graduates

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Abstract

Background: Medical practitioner is expected to have skills and knowledge in his field along with legal aspects of practise of Medicine, various medico legal issues and management of such issues. The study will help us to know the areas of weakness with regard to handling of medico legal issues among the medical graduates and can suggest remedial measures to improve the learning process.

Method: Data will be collected by giving a printed questionnaire which consists of seventeen questions related to basic knowledge of medico legal issues and its management. This will be done only after taking consent. The participants are required to answer by agreeing or disagreeing the statements given or by giving simple answers to the questions. Data will be analysed using Microsoft excel & SPSS Software and results will be presented with frequency and percentage and will be illustrated within charts and tables. 120 medical graduates participated in the study and study showed an existence of gap in the learning and practical knowledge about medical legal issues and its practise.

Conclusion: Regular training to update the knowledge of medico-legal issues is necessary to ensure continuous improvement of the quality of health care delivery and better administration of justice.

Key words: Forensic Medicine, Medico legal awareness, Medico legal issues

Introduction

A Medico-legal case is a case of injury or illness where the attending doctor, after eliciting history and examining the patient, thinks that some investigation by law enforcement agencies is essential to establish and fix responsibility for the case in accordance with the law of the land. (1) The decision to register any case as a medico legal case rests solely with the doctor who attends the case. A doctor must always exercise his judgement independently and with complete objectivity. To exercise this judgement, it is desirable that every medical practitioner should know various offences affecting human body as per law of the land. It is also desirable for the medical practitioners to know various acts, rules regulations and protocols related to procedures to be followed in the conduct of each and every medico-legal examination. In India these topics are included in the curriculum of Forensic Medicine and Toxicology.

This subject is currently taught during the second phase of MBBS course and extends for a period of eighteen months. Once they complete this phase they enter in to the final phase of MBBS course which is of twenty four months. When the medical students are graduated they are expected to judge, handle and follow the protocol for the medico legal cases based on their knowledge they acquired during their second phase of medical course.

A Registered Medical Practitioner has legal, ethical and moral responsibility to the society in their day to day clinical practise. So a medical practitioner is expected to have skills and knowledge in his field along with good ethical standards and legal awareness. (2) Lack of knowledge of Legal Medicine and legal aspects of practise of Medicine is an important issue that needs to be addressed. Hence a study is planned among the medical graduates who are getting trained in Jubilee Mission Medical College & Research Institute, Thrissur,
in Kerala.

Success of health care system not only depends on the medical knowledge, skills and attitude, also includes awareness on the legal, ethical and moral responsibility to the society. It is also pertinent that medical Profession is guided by various legal statutes. Ignorance of such legal statutes is no excuse for any dereliction of duty. Teaching of various legal statutes related to medical field, medico legal issues, protocols and guidelines to manage medico legal issues is required to equip medical practitioners with adequate knowledge which enables them to deal with various medico legal issues.\(^3,4,5\) This will help in application of medical knowledge in administration of justice. The present study is to assess the awareness related to handling of medico legal issues among the health care providers, which reflects both quality of care and administration of justice.

**Objective of the study:**

Objective of the study was to assess the awareness of medical graduates regarding medico legal aspects of clinical practise, common medico legal issues and guidelines and protocols related to such issues.

**Materials & Methods**

Descriptive Cross sectional questionnaire based study

Place of study: Jubilee Mission Medical College & Research Institute, Thrissur

**Inclusion Criteria:** Medical graduates completed Compulsory Rotatory residential Internship programme junior residents, Tutors and demonstrators.

Exclusion criteria: CRRI trainees, MBBS students.

**Data Collection and analysis:**

Data was collected by giving a printed questionnaire which consisted of seventeen questions related to basic knowledge of medico legal issues and its management. This is done only after taking consent. The participants were required to answer by agreeing or disagreeing the statements given or by giving simple answers to the questions. Data was analysed using Microsoft excel & SPSS Software and results are presented with percentage and illustrated with tables.

**Results**

A total of 120 medical graduates were participated in the study. Out of the 120 graduates, 48 participants are doing their post-graduation. All 120 participants responded to all the questions in the proforma and the percentage of correct and wrong responses are shown in Table-1.

**Table-1: Knowledge on Medico legal issues.**

<table>
<thead>
<tr>
<th>NO</th>
<th>Question</th>
<th>Number of correct responses</th>
<th>%</th>
<th>Number of wrong responses</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>There is no stipulated time period beyond which an MLC cannot be registered</td>
<td>49</td>
<td>40.83</td>
<td>71</td>
<td>59.17</td>
</tr>
<tr>
<td>2</td>
<td>The decision to register any case as a medico legal rests with police and patients or his attendants.</td>
<td>76</td>
<td>63.33</td>
<td>44</td>
<td>36.67</td>
</tr>
<tr>
<td>3</td>
<td>All brought dead cases to the hospital need to be informed to the police by the attending doctor</td>
<td>77</td>
<td>64.17</td>
<td>43</td>
<td>35.83</td>
</tr>
<tr>
<td>4</td>
<td>Minimum age for giving consent for physical examination</td>
<td>25</td>
<td>20.83</td>
<td>95</td>
<td>79.17</td>
</tr>
</tbody>
</table>
Table-1: Knowledge on Medico legal issues.

<table>
<thead>
<tr>
<th>Question</th>
<th>Correct</th>
<th>Wrong</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>When a 16 year old survivor of an alleged case of sexual assault is brought to hospital which agency is to be informed by the duty medical officer</td>
<td>45</td>
<td>37.50</td>
<td>75</td>
</tr>
<tr>
<td>When a case is examined for certification of drunkenness, Consent is REQUIRED/NOT REQUIRED if he is under arrest with a request for examination from a police officer not below the rank of Sub Inspector.(Circle the correct answer)</td>
<td>69</td>
<td>57.50</td>
<td>51</td>
</tr>
<tr>
<td>When an emergency procedure is required to save the life of a person who is unable to give consent (like unconscious person), whether the doctor will be legally protected if he performs the procedure without the consent of patient?</td>
<td>107</td>
<td>89.17</td>
<td>13</td>
</tr>
<tr>
<td>If a medical officer under the influence of alcohol treats a patient, it amounts to…………...</td>
<td>66</td>
<td>55.00</td>
<td>54</td>
</tr>
<tr>
<td>While collecting blood sample from a person to estimate blood alcohol level, spirit is routinely used as an antiseptic</td>
<td>92</td>
<td>76.67</td>
<td>28</td>
</tr>
<tr>
<td>Routine samples to be preserved while treating a suspected case of poisoning are</td>
<td>76</td>
<td>63.33</td>
<td>44</td>
</tr>
<tr>
<td>While performing termination of pregnancy (as per MTP Act 1971), consent of pregnant woman alone is sufficient if she is above the age of 18 years and mentally normal.</td>
<td>94</td>
<td>78.33</td>
<td>26</td>
</tr>
<tr>
<td>Passive Euthanasia is legally permitted in India</td>
<td>30</td>
<td>25.00</td>
<td>90</td>
</tr>
<tr>
<td>When a medico legal case has been registered in a hospital, a fresh registration is not required in the institution where the patient is referred to for further management</td>
<td>60</td>
<td>50.00</td>
<td>60</td>
</tr>
<tr>
<td>Consent from the patient is mandatory during preparation of wound certificate by a medical officer</td>
<td>63</td>
<td>52.50</td>
<td>57</td>
</tr>
<tr>
<td>Which official is intimated for recording dying declaration</td>
<td>81</td>
<td>67.50</td>
<td>39</td>
</tr>
</tbody>
</table>

Questions to assess the usefulness of curriculum in the subject of Forensic Medicine & Toxicology were also included in the study. The opinion of the Medical graduates are shown in Table.2
Table-2: Opinion on curriculum

<table>
<thead>
<tr>
<th>No</th>
<th>Question</th>
<th>Yes</th>
<th>%</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Is the curriculum you underwent sufficient to impart necessary information and awareness in managing common medico legal problems in day to day practise?</td>
<td>82</td>
<td>68.33</td>
<td>38</td>
<td>31.67</td>
</tr>
<tr>
<td>2</td>
<td>In the newly implemented Competency Based Curriculum, the subject of Forensic Medicine &amp; Toxicology has been moved to final phase of MBBS course. Will it be helpful to the medical graduates for better alignment of medico legal knowledge and clinical practise?</td>
<td>105</td>
<td>87.50</td>
<td>15</td>
<td>12.50</td>
</tr>
</tbody>
</table>

Discussion

Registration of Medico legal cases:

There is no stipulated time period beyond which an MLC cannot be registered is a false statement but more than 50 per cent of the doctors responded to this statement by saying there is a fixed time for registration of medico legal cases. The decision to register any case as a medico legal rests with police and patients or his attendants is a wrong statement. The decision to register any case as medico legal rests solely with the doctor who attends the case. This was not known to 36.67 per cent of graduates. These two basic concepts in medico legal cases shall be known to each and every medical practitioner. When a medico legal case has been registered in a hospital, a fresh registration is not required in the institution where the patient is referred to for further management. Only 50 per cent doctors responded like this. The only duty of registered medical practitioner is a mention must be made in admission document that the case is already registered in the previous institution. If the case is not registered in the first hospital a copy of the reference letter shall be attached along with the medico gal certificate/ report.

Brought dead to hospital

All brought dead cases to the hospital need to be informed to the police by the attending doctor. The time and date on which dead person was brought to the hospital, name of the accompanying person and alleged history shall be noted. It’s the duty of the attending doctor to send the intimation immediately to the police and transfer the dead body to the mortuary. 35.83 per cent doctors considered this need not be informed to legal authority.

Consent for physical examination:

Only 20.83 per cent participants knew the minimum age for giving consent for physical examination is 12 years. As per section 89 of Indian Penal Code a Child below the age of 12 years and a person with mental illness cannot give a valid consent to suffer any harm which may result from an act done in good faith and for its benefit. (6)

Reporting in sexual assault cases

When a 16 year old survivor of an alleged case of sexual assault is brought to hospital which agency is to be informed by the duty medical officer was a question asked in the study, only 37.50 graduates responded to this correctly. As per section 19 of the protection of Children from sexual offences act 2012, any person who has apprehension that an offence under this act is likely to be committed or has knowledge that such an offence has been committed, he shall provide such information.
Examination of an arrested person:

Whenever an accused person under arrest is brought for the conduct of a medico legal examination with a requisition from a police officer not below the rank of a Sub inspector of police and such person refuses consent for the said medico-legal examination, the medical officer should examine the person even using reasonable force, as per provisions of Sec.53 of Cr.P.C. This is correctly given only by 57.50% medical graduates participated in the study.

Consent during emergency

When an emergency procedure is required to save the life of a person who is unable to give consent (like unconscious person), whether the doctor will be legally protected if he performs the procedure without the consent of patient? The answer is yes and response from 89.17 doctors was found correct. As per Section 92 of Indian Penal Code, consent is absolutely dispensed with when the circumstances are such as to render consent is not possible or when, in case of person incapable of assenting, there is not one at hand whose consent can be substituted. (8)

Infamous conduct

If a medical officer under the influence of alcohol treats a patient, it amounts to Infamous conduct. 45.00 per cent medical graduates depicted this as Medical Negligence.

Samples in poisoning cases

Medical Officers should be aware of the importance of preserving the material objects like stomach aspirate, vomitus particles, blood, urine, faeces, remnants of food and medicine etc.. the examination of which may help to identify the injurious agent, in cases where such facts may have to be established in a Court of Law at a later stage. 63.33 per cent doctors correctly mentioned at least useful samples for poison detection. While collecting blood sample from a person to estimate blood alcohol level, spirit is routinely used as an antiseptic is a false statement and responded correctly by 76.67 per cent doctors. While collection of blood, the solution used to disinfect is iodine, ether or seventy per cent ethyl alcohol, the sample may be mildly contaminated the sample and can give a deceptive high value. (9)

MTP and consent

While performing termination of pregnancy as per MTP Act 1971, consent of pregnant woman alone is sufficient if she is above the age of 18 years and mentally normal. This is a true statement and is given correctly by 78% of doctors.

Euthanasia in India

Even though Passive Euthanasia is legally permitted in India by a recent verdict by the Supreme Court, 75 per cent of participants said it is not permitted legally in India. The intent to kill makes euthanasia as a crime under the Indian Penal Code till recently. But Supreme court in a landmark judgement on 9 March 2018 declared the right to die with dignity as a fundamental right and passed an order allowing passive euthanasia in the country and issued guidelines in recognition of “living will” made by terminally-ill patients.

Certification of injury

Physical examination of a person without his consent is assault except in situations specified by the Law. Hence consent should be obtained before conducting any medico-legal examination on the body of the person except in situations where the injured person directly comes to or is brought by anyone for treatment of injuries; poisoning etc. (10) 47.50 per cent graduates said consent is mandatory

Dying declaration

A dying declaration forms a basis for conviction without any corroborative evidence if it is reliable and truthful. A dying declaration recorded by a competent magistrate has a significant reliability or acceptability than oral evidence or a dying declaration recorded by the investigating officer. (11) 32.50 per cent graduates participated in this study was not aware of this concept.

Usefulness of curriculum

From the above responses of medical graduates it can be stated that many gaps exists in their knowledge
about many important aspects of their day to day clinical practise related to medico legal issues. All the concepts which reflected in the questionnaire asked in the study are covered during second phase of MBBS in the subject of Forensic Medicine and Toxicology. 68% of doctors said the curriculum they underwent is sufficient to impart necessary information and awareness in managing common medico legal problems. But the result of the study does not support this view of the graduates.

Medical Council of India in 2019 implemented the competency based curriculum with a view to create an Indian Medical Graduate possessing requisite knowledge in skills, attitudes, values and responsiveness. The subject of Forensic Medicine and Toxicology extends over a period of two and a half years during the second and third phase of MBBS. The teaching learning of this subject is aimed at producing a graduate who can demonstrate a clear understanding of medico legal responsibilities in primary and secondary sittings. The Medical graduates who participated in this study were of the view that the newly implemented Competency Based Curriculum and the extension of the subject of Forensic Medicine & Toxicology to final phase of MBBS course will be helpful to the medical graduates for better alignment of medico legal knowledge and clinical practise.

**Conclusion**

Many gaps detected in the learning of the subject of Forensic Medicine and Toxicology and medico legal awareness after graduation. The competency based curriculum in the subject of Forensic Medicine & Toxicology which is introduced in the year 2019 may reduce the gaps in the learning and practising to a great extent. The internship programme must contain training programmes to make medical graduates aware of medico legal aspects of clinical practise and also to develop skills for medico legal examination and management. The post graduates students irrespective of their subjects shall be given training programs to improve their understanding of medico-legal responsibilities.

**Limitations of the study:** The study participants are graduates from a single institution and graduates doing their post-graduation in the same institution.

**Ethical Clearance:** Clearance has been obtained from the Institutional Ethical and Research Committee prior to the study.

**Conflict of Interest:** None to be declared

**Funding and support:** None

**References**

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Effectiveness of Education and Information Technology on Menopausal Syndrome among Rural and Urban Premenopausal Women

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Abstract:

Background: Menopause is defined as the point in time when menstrual cycles permanently cease due to the natural depletion of ovarian oocytes from aging. The diagnosis is typically made retrospectively after the woman has missed menses for 12 consecutive months. Pre-menopause is a biological stage in a woman’s life when she is no longer fertile and is marked by the cessation of menstruation. The study aimed to assess the effectiveness of education and information technology on menopausal syndrome among rural and urban premenopausal women.

Methods: Pre experimental research design was used with 50 rural premenopausal women and 50 urban premenopausal women samples who matched the inclusion criteria were selected by simple random sampling technique. Demographic variables were collected by self structured questionnaires. The results of the study are in rural, 31(62%) women had adequate knowledge in pre test and 40(80%) in post test, 15(30%) women had moderate knowledge in pre test and 10(20%) in post test, 4(8%) women had adequate knowledge in pre test. In urban 42(8%) women had adequate knowledge in pre test and 39(78%) in post test, 7(14%) women had moderate knowledge in pre test and 11(22%) in post test, 1(2%) women had adequate knowledge in pre test on menopausal syndrome among rural and urban premenopausal women.

Conclusion: The study concludes that majority of the women had a negative outlook towards premenopausal syndrome and their management. After providing information regarding premenopausal syndrome and management women had a positive outlook.

Key Words: Education and Information Technology, Premenopausal Syndrome, Management and Menopause

Introduction

Menopause is defined as the point in time when menstrual cycles permanently cease due to the natural depletion of ovarian oocytes from aging. The diagnosis is typically made retrospectively after the woman has missed menses for 12 consecutive months. It marks the permanent end of fertility and the average age of menopause is 51 years. It is a natural process just as puberty is natural. Puberty prepares a girl and bear children and menopause prepares women to cease to be able to conceive. Both cause sudden changes in one’s body, puberty by introducing hormones and menopause by withdrawing them1-2.

The term menopause is derived from two Greek words ‘meno’ and ‘pause’ meaning “month” and “stop”. Thus it is perm Menopause is the permanent cessation of menstruation at the end of a woman’s reproductive life due to loss of ovarian follicular activity. It is the point of time when the last and final menstruation occurs. The clinical diagnosis is confirmed following stoppage of menstruation (amenorrhea) for twelve consecutive months in absence of any other pathology3-5.

Pre menopause is the physiological termination of normal menstrual cycles. Pre menopause is generally caused more early than the normal age which is associated with the cessation of the menstrual cycles6,7. Pre menopause occurs when the ovaries virtually stops
producing the estrogen which generally leads the fertility aspect of the women to shut down. Because pre menopause is a very important period in women’s life; informed knowledge of what to expect will go a long way to prepare a woman for what is to come during menopause proper, especially because each woman’s transition from pre menopause to menopause may differ drastically due to the difference in the amount of the male hormone androgens that may be produced, which accounts for the devastating secondary male characteristics often exhibited by menopausal women.

Perimenopause or menopausal transition is the stage of a women’s reproductive life that begins several years before menopause, when the ovaries gradually begin to produce less estrogen. It usually starts in the women’s forties. Perimenopause lasts up until menopause, the point when the ovaries stop releasing eggs. In the last one to two years of perimenopause, the decline in estrogen accelerates. At this stage, many women experience menopausal symptoms. Since menopause is due to the depletion of ovarian follicles/oocytes and severely reduced functioning of the ovaries, it is associated with lower levels of reproductive hormones, especially estrogen. Low estrogen can result in vasomotor instability (such as hot flushes and night sweats), psychological changes (such as mood swings, depression, and difficulty concentrating), insomnia, genital tract atrophy (such as vaginal dryness, painful intercourse, and urinary incontinence), and skin changes (such as thinning and decreased elasticity). Lower androgen levels (male hormones) can contribute to the loss of sex drive. Any abnormal vaginal bleeding should be reported immediately to your doctor, since this may represent a precancerous or cancerous condition of the uterus or endometrial lining.

In general, menopausal symptoms are burdensome to many women who suffer from them. Although the impact of these symptoms on health-related quality of life has been reported across a variety of populations incorporating different instruments, the burden these symptoms pose to society through increased healthcare utilization and lost productivity and wages has not been well established. The objective of this study was to evaluate the impact of menopausal symptoms on health-related quality of life and productivity and quantify the economic burden. Though studies exist on menopausal women, only a few focused on the health of these women. Midlife is thus a time to focus on oneself and to seek resources from within the family and community to maintain and enjoy equilibrium. Women need knowledge about what to expect and how to cope with changes during menopause. Hence the investigator has planned to conduct a comparative study to assess the level of knowledge and intervention on signs, symptoms and management of menopausal syndrome among rural and urban premenopausal women.

**Material and Methods**

A pre experimental study was chosen to assess the effectiveness of education and information technology on menopausal syndrome among rural and urban pre menopausal women. The study was conducted in two different settings one in Singanodai Village (rural) and another in Thirukkadaiyur (urban). 100 samples 50 samples from rural pre menopausal women and 50 samples from urban premenopausal women (40-55 years) who meet the inclusion criteria were selected by using simple random sampling technique. The data to assess the knowledge was collected by using the self structured questionnaire. The collected data were analyzed by using descriptive and inferential statistics.

**Result**

The present investigation in rural area brings about that majority of the women belong to age bunch between 46-50 years who moderately replied (90%), dominant part of them belong to Hinduism (84%), a large portion of the women had grade school education (56%), larger part of them were hitched (74%), most of them share joint family is(68%), the greater part of the ladies were now not working (76%), the vast majority of them accumulated wellspring of data from wellbeing camp (72%), majority of family income is lesser than 10000 (86%).

The present study results in urban area depicts that women with age bunch of 40-45 years answered moderately (88%), majority of them were Hindus (74%), most of the people had high school and primary school (28%), majority of them were married (72%), majority of them reside joint family (54%), most of the people were currently not working (72%), the vast majority of them accumulated source of information from community
health nurse (64%), majority of them had family income between 11000-30000 is (68%).

Table 1: Distribution of level of pre and post test knowledge and practice on menopausal syndrome among rural premenopausal women.

<table>
<thead>
<tr>
<th>Level of Knowledge</th>
<th>Rural women(N=50)</th>
<th>Rural women(N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Adequate knowledge</td>
<td>31</td>
<td>62%</td>
</tr>
<tr>
<td>Moderate knowledge</td>
<td>15</td>
<td>30%</td>
</tr>
<tr>
<td>Inadequate knowledge</td>
<td>4</td>
<td>8%</td>
</tr>
</tbody>
</table>

**TABLE 1** shows that pre test data in rural area, 31(62%) has adequate knowledge, 15(30%) has moderate knowledge and 4(8%) has inadequate knowledge. The post test data revealed that in rural area, 40(80%) has adequate knowledge and 10(20%) has moderate knowledge.

Table 2: Distribution of level of pre and post test knowledge and practice on menopausal syndrome among urban premenopausal women.

<table>
<thead>
<tr>
<th>Level of Knowledge</th>
<th>Urban women(N=50)</th>
<th>Urban women(N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Adequate knowledge</td>
<td>42</td>
<td>84%</td>
</tr>
<tr>
<td>Moderate knowledge</td>
<td>7</td>
<td>14%</td>
</tr>
<tr>
<td>Inadequate knowledge</td>
<td>1</td>
<td>2%</td>
</tr>
</tbody>
</table>

**TABLE 2** shows that the pre test data in urban area, 42(84%) has adequate knowledge, 7(14%) has moderate knowledge and 1(2%) has inadequate knowledge. The post test data revealed that in urban area, 39(78%) has adequate knowledge and 11(22%) has moderate knowledge.

Table 3: Mean and standard deviation for the effectiveness of pre-test and post-test of menopausal syndrome among rural premenopausal women. (N=100).

<table>
<thead>
<tr>
<th>Level of pre-test and post-test.</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Wilcoxon on rank sum Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre – test</td>
<td>32.2</td>
<td>7.011</td>
<td>Z= – 5.9683 W-564 P &lt;0.00001 POSITIVE (S)</td>
</tr>
<tr>
<td>Post – test</td>
<td>26.94</td>
<td>2.216</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3 Show that the overall effectiveness of pre test mean value is 32.2 and standard deviation is 7.011. Post test mean value is 26.94 and standard deviation is 2.216 and Wilcoxon rank sum test value is \( Z = -5.9683, W = 564, P < 0.00001 \), it will be Positive and significant.

### TABLE 4 : Mean and standard deviation for the effectiveness of pre – test and post - test of menopausal syndrome among urban premenopausal women.

<table>
<thead>
<tr>
<th>Level of pre-test and post – test.</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Wilcoxon on rank sum Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre – test</td>
<td>28.22</td>
<td>9.751</td>
<td>( Z = -5.7115 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( W = 473 )</td>
</tr>
<tr>
<td>Post – test</td>
<td>26.1</td>
<td>3.360</td>
<td>( P &lt; 0.00001 ) *</td>
</tr>
</tbody>
</table>

TABLE 4 Show that the overall effectiveness of pre – test mean value is 28.22 and standard deviation is 9.751. Post – test mean value is 26.1 and standard deviation is 3.360 and Wilcoxon rank sum test value is \( Z = -5.7115, W = 473, P < 0.00001 \), it will be Positive and significant.

Table 5: Association between post test knowledge and demographic variables of urban premenopausal women.

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Adequate</th>
<th>Moderate</th>
<th>Chi Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Types of family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Nuclear</td>
<td>36</td>
<td>72%</td>
<td>-</td>
</tr>
<tr>
<td>· Joint</td>
<td>10</td>
<td>20%</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 5 showed that there was statistically significant found between the types of family and the level of knowledge and intervention on menopausal syndrome among urban premenopausal women in Thirukkadaiyur.

### Discussion

The present study in rural area the pre test data shows that, 31(62%) has adequate knowledge, 15(30%) has moderate knowledge and 4(8%) has inadequate knowledge. The post test data revealed that in rural area, 40(80%) has adequate knowledge and 10(20%) has moderate knowledge. The present study in urban area the pre test data shows that, 42(84%) has adequate knowledge, 7(14%) has moderate knowledge and 1(2%) has inadequate knowledge. The post test data revealed that in urban area, 39(78%) has adequate knowledge and 11(22%) has moderate knowledge.

This study is supported by Paudyal et al. (2014) who conducted the study to assess the Knowledge on perimenopausal symptoms among women attending Lumbini Medical College Teaching Hospital his results depicts that 63.4% of respondents had poor, 33.8% had fair and only 2.8% had good level of knowledge.
regarding menopause. The reason behind the poor knowledge may be due to less appropriate source of information as majority of the women (81.2%) discuss their problems with their friends or relatives but not with doctors or nurses. Concerning hot flushes as a perimenopausal this study revealed that, almost half of the respondents (49.3%) answered that it is one of the perimenopausal symptom

According to Park and Lee, 2011 Korean postmenopausal women had more negative attitudes toward menopause and a low level of performing health promoting behaviors. Hot flush is one of the most commonly reported symptoms during menopause and it is referred to as vasomotor symptoms along with night sweats.

**Conclusion**

The study concludes that majority of the women had a negative outlook towards premenopausal syndrome and their management. After providing information regarding premenopausal syndrome and management women had a positive outlook.

**References**

Clinical Study of Combined Endoscopic And Percutaneous Management of a Late Complication Following Tension-free Vaginal Tape (TVT) Anchored Stone for Female Stress Urinary Incontinence

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Abstract

TVT is a simple and less-invasive variation of the suburethral sling with higher success rates¹. It has been rapidly gaining popularity worldwide but it has also lead to increasing number of patients presenting with associated complications. Bladder erosion through the bladder wall occurring predominantly within the first 2 years the placement of TVT is producing tension or in the close proximity of the bladder..Formation of a calculus in the urinary bladder secondary to an intravesical mesh erosion is relatively infrequent rare and delayed complication.Owing to the scarcity of the data on mesh erosion there is no consensus regarding the ideal approach to remove the mesh Combined endoscopic and percutaneous approach should be opted as the preferred approach .Open surgery should be opted only if minimal invasive approach fails.

Keywords: stress urinary incontinence, sling surgery, stone, endo urology

Introduction

TVT is a simple and less-invasive variation of the suburethral sling with higher success rates¹. It has been rapidly gaining popularity worldwide but it has also lead to increasing number of patients presenting with associated complications. Bladder erosion through the bladder wall occurring predominantly within the first 2 years the placement of TVT is producing tension or in the close proximity of the bladder..Formation of a calculus in the urinary bladder secondary to an intravesical mesh erosion is relatively infrequent rare and delayed complication.

Case 1

A 55 year old post-menopausal woman was operated and TVT applied for genuine stress urinary incontinence 6 years ago, postop course was uneventful. Patient had come with complaints of dysuria and suprapubic pain with recurrent UTI since 6 months in spite of repeated treatments with antibiotics. On evaluation USG KUB and Xray pelvis it was found to be bladder stone formed over intravesical eroded portion of sling. Intraoperative cystoscopy revealed to be a bladder stone anchored to the intravesical eroded TVT mesh. A 26Fr Amplatz sheath after serial dilation was placed percutaneously at suprapubic region under endoscopic vision. Then the suprapubic approach used to fragment the calculus was done by laser lithotripsy. After that transurethral excision of the exposed mesh was performed with holmium Yag laser & adequate traction using percutaneous suprapubic amplaz. With the use of grasping forcepely while performing cystoscopy by a rigid nephroscope in order to pull together with the intravesical eroded portion of sling out of the bladder wall. Operative time was around
25 min. Postoperative course was uneventful. Suprapubic and perurethral catheter were removed on second and seventh post operative day respectively. Patient is asymptomatic on 1 year follow up.

**FIGURE 1:** XRAY PELVIS & ULTRASONOGRAPHY SHOWS BLADDER STONE

**FIGURE 2:** ENDOSCOPIC VIEW OF TWO ENDS OF BLADDER STONE
Case 2

A 52 year old lady had stress urinary incontinence and was treated by tension-free vaginal tape 1 year before presentation. Her main complaints include persistent stress urinary stress incontinence since the early postoperative period. Gynecological examination revealed urethral hypermobility with grade III cystocele. CT(Computed tomography) Urography revealed a vesical calculus fixed to the right lateral wall of urinary bladder. On cystoscopy, an encrustation over the bladder wall is seen where the polypropylene mesh has eroded the wall. It was fragmented using pneumatic lithotripsy. The transurethral resection of the mesh and surrounding bladder mucosa was performed. The operative time was 20 min. The patient was discharged on postoperative day one. During postoperative follow-up two months later, the bladder was healed completely on cystoscopy.

Case 3

A 65 year old obese lady was referred with severe urgency, urgency urinary incontinence,dysuria, occasional hematuria and recurrent urinary tract infection to urology opd. She was operated and TVT applied for pure stress urinary incontinence 3 years ago without any complication and normal postoperative course. On sonography and Xray KUBIt showed bladder stone formed over eroded part of sling which had infiltrated the bladder. Cystoscopy revealed to be a bladder stone anchored to the intravesical eroded TVT mesh. A 24 Fr Amplatz sheath after serial dilation was placed percutaneously at suprapubic region under endoscopic vision. Then the suprapubic approach used to fragment the calculus was done by laser lithotripsy. After that transurethral excision of the exposed mesh was performed with holmium Yag laser & adequate traction using percutaneous suprapubic amplaz. With the use of grasping forcep while performing cystoscopy by a rigid nephroscope in order to pull stones together with the intravesical eroded portion of sling out of the bladder wall. Operative time was around 25 min. Postoperative course was uneventful. Operative time was around 25 min. Postoperative course was uneventful. Suprapubic and perurethral catheter were removed on second and seventh post operative day respectively. Patient is asymptomatic on follow up.

Discussion

Although TVT procedure is simple and minimally invasive procedure there are many serious complications such as bowel and bladder perforation, small bowel obstruction, retropubic hematoma, vessels injury and tape erosion in to urethra, vagina and urinary bladder. Traditionally an open cystoraphy approach is preferred during which a wide excision of the mesh is performed. However, various endourological approaches have been performed in the last decade which includes conventional laparoscopic transvesical single-port procedures, transurethral procedures and suprapubic-assisted transurethral procedures.

An isolated transurethral approach represent a lease invasive method but its association with residual mesh in the detrusor is high which may lead to irritative voiding symptoms secondary to urinary tract infection. Usage of Single-port laparoscopic approach had been reported to provide a “radical” excision but being a more invasive and certainly more complex than a transurethral approach is a disadvantage. A safe, simple, minimally invasive, successful treatment option is Combined endoscopic and percutaneous approach in which radical removal can be done with minimal bladder damage. It is alternative to both the transvaginal and transabdominal approach to extract stone and excising intravesical mesh.
Conclusion

Owing to the scarcity of the data on mesh erosion there is no consensus regarding the ideal approach to remove the mesh. Combined endoscopic and percutaneous approach should be opted as the preferred approach. Open surgery should be opted only if minimal invasive approach fails.

Ethical Clearance - Taken from sumandeep vidyapeeth institutional ethics committee

Source of Funding - Self

Conflict of Interest - Nil.

References


Medicolegal Evaluation of Burn Injuries- An Autopsy Based Study

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\(^1\)Assistant Professor, FMT, Hitech Medical College, Bhubaneswar, Odisha, \(^2\)Associate Professor, FMT, SCB Medical College, Cuttack, Odisha, \(^3\)Assistant Professor, FMT, SCB Medical College, Cuttack, Odisha

Abstract

Even before the primitive man learnt to use fire, he has been a victim of it. Our study was carried out over a period of 2 years starting from 1\(^{st}\) September 2012 to 31\(^{st}\) August 2014 at SCB Medical College, Cuttack, Odisha on burn deaths. Aim and objective of the study was to know demographic pattern, cause of death and relation of body surface involved.

The rate of burn death was 16.7\% of the total autopsy. Female victims outnumbered male victims with a female to male ratio approximately 3.2 : 1. Majority of the cases belong to adolescent and young adult (11-40 years) age group. Maximum no. of victims were married. In most of the cases fatal period was within 24 hours. Most of the deaths occurred when >40\% of TBSA is involved. Most of the victims died within 1 week. More than 80\% of TBSA are involved in majority of cases of shock, whereas a wide range of TBSA(30-100\%) is involved in cases of septicaemia. Major cause of death in burn was septicaemia.

Key words: Burn, demography, cause of death, body surface involved

Introduction

A Burn is an injury which is caused by application of heat or chemical substances to the external or internal surfaces of the body, which causes destruction of tissues\(^9\). Thermal burns and related injuries are a major cause of death and disability affecting the entire world and more so to the developing countries like India. According to The estimated annual burn incidence in India is approximately 6-7 million per year. The high incidence is attributed to illiteracy, poverty and low level safety consciousness in the population\(^15\).

The problem of burn in developing countries like India, are largely related to the nature of domestic appliances used in our country. Conventional methods like using wood, leaves, straw, open chullha, kerosene stove, kerosene lamps, LPG and natural gas stove are used for cooking, heating and lighting purpose. Various socio-cultural factors present in our country also contribute at times. In our society, accidental burns in women occur commonly, which they are more vulnerable to, as most of the women (housewives) spend their time in the household especially in the kitchen. Mortality due to burn injuries is higher because of lack of awareness among people and lack of availability of health care services.

Suicidal trend is also increasing in our country due to many socio-economic factors like abolition of joint family, domestic quarrel, mental depression due to failure in exam/love, drug addiction, mental illness, chronic disease and disability etc. Sometimes people resort to criminal act like murder, rape and to conceal the crime, they burn the body to report it as a post-mortem burn. Rapid industrialization and increasing fire disasters due to many causes are other factors contributing to the burden of disease in developing countries like India. Many established factors decide the fate of burn affected patients, but still some hidden factors are there which decide their mortality. Assessment of outcome in burn patients following admission to a burn centre may be
useful to determine the requirements of nursing care, diagnostic modalities, medical treatment, surgical interventions and rehabilitation therapy.

**Aim and Objective**

1. To determine the demographic distribution of burns.
2. To study the various causes of death in burn injuries.
3. To link the survival rate to the body surface involvement.

**Material and Method**

This study was conducted at S.C.B Medical College & Hospital, Cuttack, Odisha during the period of September 2012 to August 2014. In this study all burn cases brought to the Central Morgue, SCBMCH, Cuttack irrespective of alleged manner were taken into consideration. Case history was taken from the attendant in Burn Unit as well as from the attendant of the deceased, accompanying police personnel, inquest report & dead body challan in the mortuary during autopsy. In this study, the treatment record as well as all the necessary laboratory investigations there in are also taken into consideration. The detail case history, circumstantial evidence where available, treatment records, laboratory investigation reports, autopsy findings were analysed and compared.

**Exclusion Criteria**

Instantaneous deaths due to burns involving muscles, bones, showing features of charring as well as features of post-mortem burns are excluded from the study. Also all the Paediatric burn cases admitted to the Department of Paediatrics, S.C.B Medical College & Hospital are excluded from this study.

**Results**

A total of 301(16.7%) cases of burns had been reported out of 1799 cases of all types of autopsies. Sex wise distribution of burn cases in the study period wherein the females represent 230(76.4%) cases and males represent 71(23.6%) cases. The average female to male ratio is found to be 3.2:1.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>4 (5.6%)</td>
<td>7 (3%)</td>
<td>11 (3.6%)</td>
</tr>
<tr>
<td>11-20</td>
<td>7 (9.8%)</td>
<td>72 (31.3%)</td>
<td>79 (26.2%)</td>
</tr>
<tr>
<td>21-30</td>
<td>26 (36.6%)</td>
<td>94 (40.8%)</td>
<td>120 (39.8%)</td>
</tr>
<tr>
<td>31-40</td>
<td>19 (26.7%)</td>
<td>28 (12.2%)</td>
<td>47 (15.6%)</td>
</tr>
<tr>
<td>41-50</td>
<td>9 (12.6%)</td>
<td>12 (5.2%)</td>
<td>21 (7%)</td>
</tr>
<tr>
<td>51-60</td>
<td>3 (4.2%)</td>
<td>8 (3.4%)</td>
<td>11 (3.6%)</td>
</tr>
<tr>
<td>61-70</td>
<td>2 (2.8%)</td>
<td>8 (3.4%)</td>
<td>10 (3.3%)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1 (1.4%)</td>
<td>1 (0.4%)</td>
<td>2 (0.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>71 (100%)</td>
<td>230 (100%)</td>
<td>301 (100%)</td>
</tr>
</tbody>
</table>
Tab.1 depicts the incidence of burn cases according to age and sex. 81.6% of the total cases belong to adolescent and young adult (11-40 years) age group. The peak incidence is observed in the age group 21-30 years involving 120(39.8%) victims, followed by 11-20 years comprising of 79(26.2%) victims. On sex wise analysis, the peak incidence in males is found to be in 21-30 years age group, comprising 26 (36.6%) cases followed by 19(26.7%) cases in 31-40 years age group. In females also the sufferers are mostly encountered from 21-30 years age group comprising 94 (40.8%) cases, followed by 11-20 years age group accounting 72 (31.3%) of the cases. This indicates that the susceptible age group for both male and female is 21-30 years.

Tab.2: Marital Status

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>40(56.3%)</td>
<td>171(74.3%)</td>
<td>211(70%)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>31(43.7%)</td>
<td>59(25.7%)</td>
<td>90(30%)</td>
</tr>
<tr>
<td>Total</td>
<td>71(100%)</td>
<td>230(100%)</td>
<td>301(100%)</td>
</tr>
</tbody>
</table>

Tab.2 above represents the marital status of the sufferers. 70% of burn victims are married and 30% are unmarried. On sex wise distribution, maximum sufferers are married in both sexes comprising 171(74.3%) females and 40(56.3%) males.

Tab.3: Duration of Survival

<table>
<thead>
<tr>
<th>Duration of Survival</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot Death</td>
<td>10(14.1%)</td>
<td>17(7.4%)</td>
<td>27(9%)</td>
</tr>
<tr>
<td>&lt;12 hrs</td>
<td>14(19.7%)</td>
<td>49(21.3%)</td>
<td>63(20.9%)</td>
</tr>
<tr>
<td>12-24hrs</td>
<td>10(14.1%)</td>
<td>34(14.8%)</td>
<td>44(14.6%)</td>
</tr>
<tr>
<td>2-3days</td>
<td>9(12.7%)</td>
<td>24(10.4%)</td>
<td>33(11%)</td>
</tr>
<tr>
<td>4-7days</td>
<td>12(16.9%)</td>
<td>45(19.6%)</td>
<td>57(18.9%)</td>
</tr>
<tr>
<td>1-2weeks</td>
<td>8(11.3%)</td>
<td>39(17%)</td>
<td>47(15.6%)</td>
</tr>
<tr>
<td>&gt;2weeks</td>
<td>8(11.3%)</td>
<td>22(9.6%)</td>
<td>30(10%)</td>
</tr>
<tr>
<td>Total</td>
<td>71(100%)</td>
<td>230(100%)</td>
<td>301(100%)</td>
</tr>
</tbody>
</table>
Tab.3 describes the survival period of the burn victims. Most of the victims 224(74.4%) died within 1 week of the incidence, among which 134(44.5%) cases died within 24 hours. This indicates that, in most of the cases fatal period is within 24 hours.

Tab.4—Considering the fatal body surface area involvement to be 40% or more, 290 cases out of 301 are selected for analysis of TBSA.

<table>
<thead>
<tr>
<th>% TBSA Burnt</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30</td>
<td>0(0%)</td>
<td>2(0.9%)</td>
<td>2(0.7%)</td>
</tr>
<tr>
<td>31-40</td>
<td>6(9.7%)</td>
<td>5(2.2%)</td>
<td>11(3.8%)</td>
</tr>
<tr>
<td>41-50</td>
<td>7(11.3%)</td>
<td>21(9.2%)</td>
<td>28(9.7%)</td>
</tr>
<tr>
<td>51-60</td>
<td>4(6.4%)</td>
<td>18(7.9%)</td>
<td>22(7.6%)</td>
</tr>
<tr>
<td>61-70</td>
<td>8(12.9%)</td>
<td>20(8.8%)</td>
<td>28(9.7%)</td>
</tr>
<tr>
<td>71-80</td>
<td>9(14.5%)</td>
<td>21(9.2%)</td>
<td>30(10.3%)</td>
</tr>
<tr>
<td>81-90</td>
<td>11(17.7%)</td>
<td>45(19.7%)</td>
<td>56(19.3%)</td>
</tr>
<tr>
<td>91-100</td>
<td>17(27.4%)</td>
<td>96(42.1%)</td>
<td>113(39%)</td>
</tr>
<tr>
<td>Total</td>
<td>62(100%)</td>
<td>228(100%)</td>
<td>290(100%)</td>
</tr>
</tbody>
</table>

Tab.4 represents the percentage of total body surface area involved in burn deaths. In majority of the burn deaths 113(39%) more than 90% TBSA is involved, followed by 56(19.3%) death when 81-90% TBSA is involved. In 277(95.5%) cases, percentage of burn is >40% TBSA. 13(4.5%) cases are found with percentage of burn < 40% TBSA.

In male, majority of victims 17(27.4%) are found with involvement of 91-100% TBSA followed by 11(17.7%) cases where 81-90% TBSA is involved. 6(9.7%) cases are found with percentage of burn < 40% TBSA.

In female majority of victims 96(42.1%) are found with involvement of 91-100% TBSA followed by 45(19.7%) victims where 81-90% of TBSA is involved, and least number of victims 7(3.1%) where <40% TBSA is involved.
Tab.5: Distribution of survival period according to TBSA involved

<table>
<thead>
<tr>
<th>Duration of survival</th>
<th>TBSA Involved</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤30%</td>
<td>31-40%</td>
</tr>
<tr>
<td>Spot death</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&lt;12 hrs</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>12-24hrs</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-3days</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4-7days</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1-2weeks</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&gt;2weeks</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

Above table represents the relation between TBSA involved in burn injuries and duration of survival. Most of the victims 208(71.7%) died within 1 week when >40% of TBSA is involved. 18(6.2%) cases died at the spot where >90% of TBSA is involved.

Tab.6: TBSA involved and Cause of Death

<table>
<thead>
<tr>
<th>Period of survival</th>
<th>TBSA Involved</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤30%</td>
<td>31-40%</td>
</tr>
<tr>
<td>Neurogenic shock</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hypovolaemic shock</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>
Tab.6 depicts the relationship between total body surface area involved and cause of death. More than 80% of TBSA are involved in majority of cases of shock, whereas a wide range of TBSA (30-100%) is involved in cases of septicemia.

**Discussion**

The reason for gradual rise in burn death cases could be due to increase in population, lack of awareness and other social as well as environmental factors.


Females are more prone to the burn incidences because of their involvement in kitchen work. Moreover in a developing country like India, females are married earlier than males in the family and are exposed to social and family stress much earlier than males. The other reasons which contribute significantly for female preponderance are traditional clothing pattern in Indian women, illiteracy, lack of awareness, mental stress, suicidal and dowry deaths.


The reason for maximum sufferers encountered from the age group 21-30 years followed by the age group 11-20 years may be due to the fact that it is the productive age, when they are generally active and exposed to hazardous situations both at home and work. Females in this age group are mostly engaged in cooking and wear clothes like sarees, dupatta which catch fire easily.


The triggering factors for suicidal burns in married females could be young age at the time of marriage combined with inability to cope with the physical and psychological stress of marriage, harassment from in-laws. Accidental burns may be due to inadequate precautions during cooking and wearing of the loose clothes like sari. Burn as a convenient means of bride killing also contributes towards the higher incidence in the said age group and sex.

On analysing the data further, and concentrating on the duration of marriage of the married females, it is observed that majority of deaths occurred within 7 years of marriage and among them maximum within the first three years of marriage.


From above presentation it is found that most of the fatality occurred when >40% of TBSA is involved and the percentage of burn surface area increasing the duration of survival is decreasing.
While relating total body surface area involved to the cause of death we found more than 80% of TBSA are involved in majority of cases of shock, whereas a wide range of TBSA (30-100%) is involved in cases of septicaemia. As regards the cause of death, maximum number of victims died as a result of Septicaemia followed by Neurogenic shock and Hypovolaemic shock. Jain R et al (2011)\textsuperscript{10} and Gupta R et al (2012)\textsuperscript{6} agree with our study. But Dasari H et al (2008)\textsuperscript{5}, Kaulapur V V et al (2011)\textsuperscript{12}, Chawla R. et al (2011)\textsuperscript{4} reported dissimilar results as compared to our study.

The finding of the present series depicts septicaemia as the most important factor for the cause of death as it cause devitalisation of tissue leaving extensive raw areas, which usually remain moist due to the outflow of serous exudates. The exposed moist area along with the dead and devitalized tissue provides the optimum environment favouring colonisation and proliferation of numerous microorganisms, which is further enhanced by the decrease of the immune response.

**Conclusion**

In our study, we observed that most of the victims are married females of younger age group. Majority of the victims died due to septicemia within 1 week of hospitalization with burns involving more than 40% of the total body surface area. Each factor has got some influence on the fatality of the burnt victims. However, the factors like feminine gender of younger age group and most importantly the percentage of burn are the prime factors which are mainly responsible for mortality in the burn victims. Development of burn wound infections, indicating the decrease in natural defences responsible for counteracting them, is another major factor influencing the mortality of patients.

**Conflict of Interest:** None declared

**Funding:** Self

**Ethical Clearance:** Taken from Hospital Ethical Committee at SCB Medical, College, Cuttack, Odisha.

**References**


Eating Attitudes Test Students Using EAT-26 Questionnaire among Physiotherapy Undergraduate Students

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Abstract

Introduction: Increased number of high BMI individuals eventually leads them to low self-esteem, low self-evaluation which can lead to self-destructive behaviours such as uncontrollable dieting or overeating. Thus, in order to evaluate the eating attitudes a study is conducted among the undergraduate students. Incidence of fast food is increasing rapidly day by day especially in the young individuals, with improper food eating habits at irregular intervals which includes increased intake of carbonated soft drinks in young individuals, due to which there is increased risk of various health hazards out of which the most commonest health hazard is increased risk of obesity in young population especially of urban region obesity also affects the individuals in different aspects such as lethargy, psychological disturbances and physical inactivity leading to increased risk of cardiovascular complications. EAT-26 questionnaire is a screening measure which helps in determining whether you might have an eating disorder that needs professional attention. This questionnaire is a screening measure and is not designed to make a diagnosis of an eating disorder or to replace the place of a professional consultation.

Objective: The objective of the study was to get the number of individuals at risk and to analyse the change in the food habits.

Study design: The design of the study is Observational with 1 month study duration.

Result: 44 students were having inappropriate eating disorder whereas 156 students were having normal eating habits.

Conclusion: 22% of students are at risk of eating disorder tested by EAT 26 questionnaire.

Keywords: EAT-26, eating habits, fitness, Health risks.

Introduction

Increased number of high BMI individuals eventually leads them to low self-esteem, low self-evaluation which can lead to self-destructive behaviours such as uncontrollable dieting or overeating. The components of contention and impulsivity were drawn as forecasting aspects of losing weight in therapeutic participants and the degree of inhibition and counter-regulation of social behaviours is related to the severity of obsessive unhealthy eating issues. Thus, in order to evaluate the eating attitudes a study is conducted among the undergraduate students. A maladaptive perfectionism should be checked for disorder in eating attitudes for anorexia nervosa and bulimia nervosa symptoms.

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Incidence of fast food is increasing rapidly day by day especially in the young individuals, with improper food eating habits at irregular intervals which includes increased intake of carbonated soft drinks in young individuals, due to which there is increased risk of various health hazards out of which the most commonest health hazard is increased risk of obesity in young population especially of urban region obesity also affects the individuals in different aspects such as lethargy, psychological disturbances and physical inactivity leading to increased risk of cardiovascular complications. Life expectancy, honors thesis and body mass were big determinants related to dysfunctional dietary behaviour. The greater the body mass, the greater the chance that eating patterns could have become irregular. The food habits and the usage of soft drinks are at high risk to affect the lives of the young individuals in almost every aspect of life including the most important thing at the young age i.e. productivity. Nutrition consumption is linked with compulsive overeating rising in incidence. The prevalence of food addiction range is based on the criteria including age, and level of body weight. Emotions like frustration, anxiety, disappointment, and joy trigger the feeding reactions like consuming pace, food taste, and digestion. Psychological mindset has major impacts on consuming habits and behaviors, as feelings impair the regulation of fundamental processes of action and cognitive mechanisms.

Thus, in order to assess or evaluate the pattern of eating attitudes in young individuals their different tools/scales out which an EAT 26 QUESTIONNAIRE screening tool is used in order to assess the ‘eating disorder risk’ among the undergraduate physiotherapy student.

ED physiology tends to be overrepresented as opposed to the general majority of individuals with mental disorders. For people with neurodevelopmental disorders the demographic distribution for EDs is indeed not twice as significant as in the community at large. Originally designed for AN subjects, EAT represents mainly the AN prodromal symptoms, which reflects, in part, the comparably low results in the research analysis, with most of the respondents, particularly men and women, who are of ideal weight or overweight / anorexic. It is important to improve awareness about the degree and complexity of the difference among ED and ESSENCE towards being flexible to cope and establish care plans particularly for such clients. There seems to be an inadequate work in to the overlaps among ESSENCE and ED for adolescents and young adults. Diet dysfunctions, assessed with EAT, were much more prevalent and present than what was seen in the wider population, whereas in older EDs were far more severe. In the present ESSENCE sample, a substantial majority of instances had a dietary psychopathy without meeting the conventional ED requirements; nevertheless, these individuals may experience from their distressed feeding and require medical attention for a variety of reasons. In response to the claim, the EAT ratings showed that the amount of potential sub-threshold instances was slightly higher than expected from preceding research.

A practitioner with maladaptive eating habits or an ongoing unhealthy lifestyle (ED) may adapt nutritional treatment for his or her personal convictions on diet and lifestyle, thus jeopardizing evidence-based practice and, possibly, the safety and quality of care under their treatment. Past findings on this demographic have produced inconsistent outcomes, with others suggesting a greater incidence of disordered eating among learners in nutrition and dietetics compared to students undertaking certain degrees.

The screening for the eating disorder is based on the assumption that early detection can lead to early treatment to reduce the serious complications. Unhealthy food intake are characterized by a broad variety of dietary disturbances, including nutritional and nutrition problems, nutrition limits, and unsanitary and excessive obesity treatment practices.

**Methodology**

The clearance from institutional ethics committee was obtained. 200 students from the Ravi Nair Physiotherapy College were selected for the study as per the inclusion and exclusion criteria. The participants were informed about the study and the students who were willing was asked to fill the EAT-26 questionnaire. The questionnaire link was shared with the participants. After the students filled the form the results were analysed.
Procedure

The permission from the Head of Institution was obtained for the study and further permission was obtained from the institutional ethics committee. Then, the selection of the target population was done according to the inclusion and exclusion criteria. The participants who were willing to participate were explained about the study and the EAT-26 questionnaire was shared with them, after that the statistical analysis was carried out. (as shown in the flow chart)
Results

According to the feedback received from students it has been found that 44 students were having inappropriate eating habits whereas 156 students were having normal eating habits. (as shown in the pie chart)

![Pie chart showing 22% inappropriate eating habits and 78% normal eating habits]

I. EAT-26 results interpretation

Discussion

A huge mental parameter that intervenes in promoting bad eating habits has proven to be self-esteem for both males and females. Critical thinking, which is nonetheless strongly associated with fortitude, has proven unbiased and has no influence on bad eating behaviours.

Trying to cope with socio-cultural patterns is definitely a big part of the pressures of everyday, needing creation of responsive resources and toughness. The possible second leading up to the incident binary that describes poor dietary sentiments in emerging teenagers was identified as intellectual ability, defined as a trait, skill, or ability to detect and word orally and in writing expressed feelings, and control its own sentiments, i.e. explicitly suppressing them or focusing entirely on new people. Irrational role entails spree wanting to eat that is flawed to health assistance, even sometimes participating in food addiction. Yes, distractibility is also linked to people consuming externally. Nevertheless, the specific set of addictive feeding demeanour – emotional feeding, both in women and people, is often closely tied.

Along with a comprehensive article, roughly 10–30 more for each cent of the respondents struggle from hygiene-impacting personality traits and climates that urgently require the attention of the government and health care professionals. Dietary patterns in this research include ideas, values, emotions, habits, and diet relationships. Interpretation of muscle mass focuses on the individual mass assessment as ‘malnourished’ or ‘normal BMI’ or ‘obese’ versus the actual body composition.

The main problem of both emerging people’s and parents’ narratives was the lack of understanding of the existence and therapeutic treatment of feeding issues across the numerous clinicians employed at all stages of care. Inferential statistics by elderly patients presents healing with binge eating as a larger trend that extends far recovering mass. Optimizing medical expertise at certain tiers is obviously vital to timely identification and prediction, it is however significant to emphasize that therapies provide a proper balance here between required intent to improve safety, physical safety and behavioural quality of life-being.

ED has a hereditary component, such as with evolutionary, personality psychology and endemic
factors, as noted in the creation. In the light of both our recent data and past data, the physical differences or personal attributes may become critical drivers defining the implications of the distorted personal relationships 12.

The severity of the effects of dietary disabilities as well as dysfunctional food choices is just the same that it has already been related to intensified distress. Individuals with eating disorders suffer elevated incidence of certain psychiatric conditions, healthcare, congenital anomalies, role-playing abnormalities, suicidal ideation, shorter life expectancy and very case fatality levels 13.

Disillusionment with the body confidence and eating problems are considered a powerful classic example of anguish all through puberty. This vital time of life is profoundly impacted by the inherent emotional feelings of anxiety, depression, despair and denial of attraction of teenagers and adults, particularly females. Rising prevalence of undernutrition has indeed been linked with accelerated urbanization and dietary change, marked by elevated intake of Western diets rich in processed sugars, added high in fat and sugar, along with insufficient physical activity. It is difficult to ascertain the intricacies of the engagement among behaviour and preferences, self-esteem perspectives, BMI, and dietary habits such as aerobic exercise by logistic regression solo, and most of it has been partly described by prior studies 14.

A large variety of unmerited weight management interventions for someone who has a shifted awareness of their mass. They can have excessive weight habits handles, such as shedding, extreme or unjustified foods prohibition, overeating and use of additives redesigning body mass. Compulsive eating traits highlighting the differences during teenage years, likely since adolescence is a physical tests era containing major teenage body modifications growing ever more self-conscious and dismissive of their kingdom 15.

Conversely, it has been seen in current history that previous reports have undoubtedly exaggerated the incidence of disordered eating of many people, and in reality it is still increasing significantly amongst males. In any culture there are unique beauty ideals for male and female, so dynamic performance where there is little consensus between the beauty picture and the desired model. Myths about body identity can contribute to a number about severe problems, involving severe depression, binge eating and poor self-esteem. It is indicated that self-esteem, delineated as some extent of worth that an individual considers for oneself, exert an intimate ties only with social mores of any civilization. As mentioned previously, poor self-image and high probability self-esteem as a potential factor for anorexia nervosa and their interrelationships are commonly evaluated 16.

Conclusion

The eating attitude assessment was used to check the eating habits and the risk of eating disorders among students which showed that 22% of students were at risk of eating disorder tested by EAT-26 questionnaire.

Conflict of Interest – Nil

Funding – Nil

Ethical Clearance: The permission from the institutional ethics committee (IEC) was obtained before the commencement of the study RNPC201904.

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Effectiveness of Active Release Technique verses Conventional Physiotherapy in Management of Upper Cross Syndrome

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Abstract

Background: In upper cross syndrome (UCS), weaker neck flexors, anterior and middle serratus and lower trapezius along with rhomboids usually develop, and tightness of the levator scapulae, pectoralis major and upper trapezius are biomechanically adapted. Active Release Technique (ART) helps to reduce discomfort and improve the range of movement. Also, Active Release Technique (ART) is a manual procedure which is also being used for other soft tissue rehabilitation as well as for the management of the scar tissues. UCS and neck pain is common with uncomfortable job postures as well as in stress and anxiety, due to which muscle dysfunction starts which can further followed by altered posture around the neck.

Aim and Objective: To evaluate the effects of Active Release Technique verses conventional physiotherapy in management of upper cross syndrome physiotherapy.

Materials and Method: 35 patients will be grouped in two groups. Both groups will be treated for six sessions. Group A will be treated with active release technique and static stretching along with hydrocollator pack. The patients coming under Group B, conventional physiotherapy would be given which include upper trapezoid stretching, levator scapulae and pectoralis major as well as rhomboid strengthening, deep neck flexors, lower trapezius along with the hydrocollator pack.

Result: The parameters of outcome measures in interval of pre and post treatment will be analysed using the statistical test namely students paired T test.

Conclusion: Based on the previous data of ART we assume it will be beneficial in relieving the symptoms involved in UCS.

Key Words: Active Release technique, Upper cross syndrome, Physiotherapy

Introduction

Neck is a body part between the head and the shoulder and it also connects the head with the body.

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The position of the neck and the bottom of the head and shoulders is at greater risk as people sit in the wrong way for long periods of time as there is a rapid rise of time span in the following activities like studying, writing or using a computer.

In upper cross syndrome (UCS), there will be weakened neck flexors, anterior and middle serratus and lower trapezius along with rhomboids, and you will observe the tightness of the levator scapulae, pectoralis major and upper trapezius. This condition is assigned cross name because it can draw “X” (a cross) across the upper body, in UCS primarily muscle imbalance occurs,
which eventually happens in tonic and phasic muscles.\textsuperscript{2}

Individuals with upper cross syndrome may exhibit the following features which can be text neck posture, rounded upper back, elevated and prolonged shoulders, winged scapulae, and reduced thoracic spine mobility. The root of symptoms in mechanical neck pain is not well known, but has been hypothesized to be linked to various anatomical structures, especially the cervical spine’s zygapophyseal or uncovertebral joint. A major reason of neck pain is awkward working posture, anxiety, fatigue, heavy lifting and physically challenging jobs.\textsuperscript{3}

Manual material handling tasks can sometimes lead to the initiation of the musculoskeletal disorders, for example, employees who do their work in an unacceptable role or in an unhealthy posture and perform the same behavior during their working day.\textsuperscript{4} UCS may lead to irregular kyphosis in thoracic spine, as well as changed glenohumeral joint, altered cervical spine biomechanics, can result in loss of cervical curve and may lead to cervical spine degeneration. Changes in musculature function, in people having UCS frequently may cause these individuals to experience chronic headaches.\textsuperscript{3} The occurrence of such disorders can be affected by various factors such as unsuitable posture at work and lack of regular exercise. Data suggests that in the shoulder-girdle and cervico-thoracic area 6-48 percent of the UCS population complain of pain.\textsuperscript{4}

Mechanical neck pain usually presents as neck and/or shoulder pain with mechanical features which may include signs triggered due to sustained postures of the neck, neck movement, or cervical muscle palpation. The root cause of mechanical neck pain symptoms is not completely recognized. Uncomfortable job posture, anxiety, exhaustion, heavy weight lifting and physically demanding jobs are typical causes of the neck pain.\textsuperscript{5} Mechanical dysfunction, which triggers unusual joint movement, is a common cause of neck pain, because abnormal cervical joint mobility inside the joint capsule can restrict neck motion. What’s more, unbalanced soft tissue around

The head and neck structure can set limits on the head’s range of motion (ROM) and cause discomfort to the neck. Therefore, once the muscle dysfunction starts, traditional muscle imbalance trends and altered posture follow.\textsuperscript{6}

Active Release Technique (ART): The Active Release Technique (ART) is a manual procedure for the soft tissue rehab requiring the removal of scar tissue which can cause or which is causing discomfort, stiffness, muscle fatigue and irregular symptoms such as mechanical muscle dysfunction, myofascia and soft tissue.\textsuperscript{6} Furthermore, in patients with a partial tendon tear. ART has been documented to be beneficial for carpal tunnel syndrome, Achilles tendonitis, and tennis elbow, all of which include soft tissue near joints in the distal body parts). ART also has effectiveness in minimizing pain and increasing ROM in patients with a partial tendon tear). Most patients with chronic neck pain experience pain and limitation of movement caused by soft tissue dysfunction.\textsuperscript{6} ART helps to reduce discomfort and improve the range of movement. Thacker D. et.al. determined the use of Active Release Technique (ART) and use of prescribed exercises and they demonstrated progress in the anterior head carriage relative to traditional physical therapy with prescribed exercises.\textsuperscript{7}

Jun Ho Kim. et.al. determined the effects of the active release technique on discomfort and cervical range of movement in cases with prolonged neck pain and result in post sessions were, the Active Release Technique (ART) showed substantial increase in each parameter of the neck joint and no notable effects were present in the conventional physical therapy management.\textsuperscript{6}

Samy S. Abu Naser. et.al. determined a knowledge-based neck pain management method and an description of neck diseases were identified and described, disease causes were sketched and disease care was given at any time possible.\textsuperscript{1}

As for now there stands a paucity of studies on the upper cross syndrome. The aim of the study is to treat the patients with the upper cross syndrome through the process of the Active Release Technique (ART) and prescribed traditional or conventional physiotherapy treatment and to compare their effects that can affect cervical flexibility and to improve the symptom parameters. The goal is to enhance flexibility by using the method called Active Release Technique.

**Objective**

To evaluate the effects of Active Release Technique verses conventional physiotherapy in management of
upper cross syndrome physiotherapy.

**Methods**

This study will be carried out at Ravi Nair Physiotherapy College (RNPC), Musculoskeletal OPD, Sawangi (Meghe), Wardha, Maharashtra, India after approval from Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences (DU).

**Study design:** Interventional study

**Study setting:** ‘Ravi Nair Physiotherapy College (RNPC), Musculoskeletal OPD, Sawangi (Meghe), Wardha, Maharashtra, India’.

**Participants:**

Inclusion criteria - i. mechanical neck pain

ii. Age group between 18-35 years

iii. Based on assessment for neck pain

Exclusion criteria- i. History of trauma or surgery in cervical region.

ii. Traumatic pain

iii. Cervical pathologies

iv. Subjects taking analgesics/muscle relaxants

v. Cervical pain with radiculopathy

vi. Non-cooperative patients

**Variables**

Outcome measures:

1. Neck Disability Index (NDI) Scale

2. Visual Analog Scale (VAS)

3. Cervical ROM

4. Length of Muscle (Pectoralis Major)

**DATA SOURCE MEASUREMENT**

1. The NDI Scale validity and reliability is high, the NDI exhibits excellent reliability (ICC = 0.88; [0.63 to 0.95]). (8)

2. The VAS has good validity and reliability score the ICC for all paired VAS scores was 0.97 [95% CI = 0.96 to 0.98]. (9)

3. Reliability score of cervical goniometer ranged from 0.999 to 0.931 for all cervical movements (r=0.999-0.931). (10)

4. The length of muscle (Pectoralis Major) will be calculated for the length of the pectoralis major muscle before the start of the treatment session and after the 6th treatment session.

**Sample Size: 35**

Group A: 18 patients will be treated with the process of the active release technique and also by static stretching.

Group B: 17 patients will be treated with the process of conventional physiotherapy which will include specific prescribed exercises.

Sampling technique is purposive sampling method as in this short duration of study finding maximum many cases of upper cross syndrome will be difficult so 35 is selected as sample size.

**Procedure**

The institutional ethics committee (IEC) clearance will be obtained before the start of the study. A proper consent will be taken from the patients and the patients will be thoroughly explained about the study. The patients who wish to participate in the study will be taken into isolation in order to respect their privacy and will be given the Visual Analogue Scale (VAS) to assess the pain before the start of the treatment and the Neck Disability Index (NDI) will be given to assess the disability present before treatment at the neck and will be categorised into either group by random sampling using envelop method which will be namely Group A and Group B.

The people placed under the group A will be managed according to the active release technique and by the static stretching along with the hydrocollator pack. The patients coming under the group B will be managed with the conventional physical therapy would include the upper trapezoid stretching, levator scapulae and pectoralis major as well as rhomboid musculature strengthening, deep neck flexors, lower trapezius along
with the hydrocollator pack with six to eight layers of towel over the hydrocollator pack.\(^3\) Active Release Technique (ART): The ART will consist of protocols on both sides for the pectorals, the levator scapulae, and the upper trapezius. The treating physiotherapist must examine the subjects for the musculature involved at each scheduled session. During a therapy session, the active release procedure will be administered once to the musculature involved for 8-10 minutes. The physiotherapist would then only perform the procedure to the musculature that was found to be affected during the examination process.\(^7\)

Static Stretching: For levator scapulae stretching, the subjects will be advised to take the seating position holding the seat with one hand to prevent depression of the shoulder, then relaxing and turning the neck to the opposite side by keeping another palm behind of the skull and gradually moving below towards the underarm. They will be asked to keep the stretch for 15-20 sec on each side, with 2-3 reps. Repeat stretching 2,3 times per session.

Participants will be supposed to be in the standing position in front of the door frame with elbow bent at 90\(^\circ\) for the pectoral muscle stretch and to lean forward without going forward. The stretch is felt around the anterior chest and is kept with around 2-3 reps per session up to 15-20 sec.

**Conventional physiotherapy:**

Exercises: Exercises which will be used for the treatment of Upper Cross Syndrome (UCS) will be as follows:

Cervical Nod: The patient will be told to lie down on the floor or to stand up against a wall, pretending to touch the back of your neck to the wall or floor behind you. It is not going to strike, but you can feel your chin tuck slightly and the head lift crown. That would almost look like you’ve got a double chin. Placing a rolled up towel behind the neck may be useful in having something to push into. This motion strengthens the muscles on the front of the neck and lengthens the muscles at the base of the head, thereby helping to establish an ideal balance of length / strength in the cervical spine (back). Perform the repetitions for 10 – 15 times.

Resistance band row: The physiotherapist should make a loop of the resistance band around anything that is about hip-height (usually a door knob is good and preferred). Then, facing the door knob or anchor point, the patient is told to keep the body tall and the elbows in (not splaying open) while the patient is using both the upper limbs (UL) to pull the resistance band towards him. Depending on the resistance band tension the patient is allowed to change the standing position. The patient will be told to bring together the shoulder blades, and to draw back the elbows. He / she will be told to raise the chest and to hold the heart engaged. He / she will be asked to perform 15-20 repetitions depending on the band’s resistance), the release will also be applied with the hot pack.

**Expected Results**

Once the study result is complete, statistical analysis will be analysed using paired T-test and presented in the form of research paper.

**Discussion**

To our knowledge, this study will be the first study to evaluate effects of the active release technique in upper crossed syndrome and also to compare the effects of active release technique with the conventional physiotherapy treatment. According to the previous studies the effects of conventional physiotherapy in management for the upper cross syndrome is proven, but to our knowledge, this will be the first study to compare the effects of active release technique with the conventional physiotherapy among UCS patients. More over this study will employ well established and widely used methods with appropriate reliability and validity to assess the pain, neck disability, cervical ROM and the length of muscle. The limitation of the study would be the active release technique may not be effective as conventional physiotherapy treatment in UCS patients to help in alleviating the neck disability and to improve the cervical ROM of the patients. Therefore, this study intends to compare active release technique and conventional physiotherapy treatment in UCS patients.

**Conflict of Interest-** None

**Funding support-** None
References


Significance of Measuring Dimensions of Lumbar Lamina and Spinal Canal – A Cadaveric Study

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Abstract

Introduction: The incidence of lumbar nerve root compression and surgeries for its treatment like laminectomy, laminotomies are increasing. Similarly, intralaminar screw insertion and use of laminar hooks for stabilization is also gaining popularity. The dimensions of lumbar lamina are of importance for designing of lumbar spinal implants used. Method: The present study was undertaken to measure vertical height of lumbar lamina and width of lumbar vertebral canal in cadavers. Lumbar spine was exposed from posterior midline approach in 20 formalin preserved cadavers. The dimensions of lumbar lamina L1 to L5 were recorded with the help of Digital Vernier Caliper after removal of soft tissue from the vertebrae. The data was analyzed using SPSS software version 23. Result: Range of transverse width of vertebral canal of L1 and L2 was 14-23 mm. It gradually reduces in L3, L4 and L5 (12-21 mm). There was no significant difference in width of vertebral canal between Male and female. Mean height of lamina at junction with transverse process at L2 was (25.7±2.8mm) which reduced from L3, L4 and was lowest in L5 (20.8±2). Mean height of lamina at junction with Spinous process increased from L1 to L2 (27.55±3.3) and decreased from L3, L4 and L5 being smallest (24.3±2.7). The gender, right and left differences for the dimensions of lumbar vertebral lamina were statistically insignificant. In 35 percent cases the spinous processes were fused which was an accidental finding. Conclusion: The data so collected will be of great significance for spinal surgeons and for designing of implant around this region.

Key words: Lumbar vertebrae, Lumbar canal, Lumbar lamina, Spinous process

Introduction

The incidence of lumbar nerve root compression and surgeries for its treatment like laminectomy, laminotomies are on the rise. Placement of laminar hooks & insertion of intralaminar screws, during surgical treatment of lumbar spine disorders, is gaining popularity. The knowledge of morphometry of lumbar vertebrae is crucial for surgeons during surgical interventions and placement of implants locally¹⁻⁹. The dimensions of lumbar lamina are also essential for designing of lumbar spinal implants. So, the present study was undertaken to measure dimensions of lumbar lamina and lumbar vertebral canal.

Material & Method

This cadaveric study was carried out at the Department of Anatomy of a medical institute in India. Ethical approval to undertake the present study was obtained from the Institution Ethical Committee (IEC). Lumbar vertebra studied in 20 formalin-fixed adult human cadavers (10 female and 10 male). The age range of the cadavers was 60–95 years (mean, 77.5 years). We included sample in study which had intact vertebral column without any previous dissection, without any surgical procedures, without any trauma pathology of
spinal cord. We exclude sample from study which had carried out any surgical procedure, any external visible deformity of the vertebral column and external sign of injury. The cadavers were placed in a prone position on a flat table with hips extended. The skin was reflected from lower thoracic to sacral region and superficial and deep muscles of the back were identified and excised. The lumbar vertebrae counting from sacrum upward and cleaned. After taking measurements of lamina of L1 to L5 posterior neural arch was excised to measured transverse width of the vertebral canal with the help of Digital Vernier Caliper. The data was analyzed using SPSS software, version 23. For comparison, the level of significance (p value) is kept at 0.05.

**Result**

On both the sides, the mean height of lamina at its junction with transverse process at L2 was found to be highest (25.7±2.8mm), which gradually found to reduce from L3, L4 and was lowest in L5 (20.8±2). Bilaterally, the mean height of lamina at its junction with Spinous process was found to increase from L1 to L2 (27.55±3.3) and decreased from L3, L4 and L5 being smallest (24.3±2.7) (Table 1).

On both the sides, the mean height of lamina at junction with spinous process at L2 was found to be highest which gradually found to reduce from L3, L4 and was lowest in L5. We did not find any statistically significant difference for dimensions of lamina between Males & Females or between right & left sides. We did not find any statistically significant difference for dimensions of lamina between Males & Females or between right & left sides (Table 2).

Transverse width of Vertebral canal is also known as interpedicular distance. The present study found that the range of transverse width of vertebral canal of L1 and L2 was 14-23 mm. It gradually reduced in L3, L4 and L5 (12-21 mm). The mean transverse width of vertebral canal was compared at all the vertebral levels between males & female using two sample T tests. There was no significant difference in width of vertebral canal between Male and female at any level (Table 3 & Fig 2).

<table>
<thead>
<tr>
<th>Vertebral Level</th>
<th>Height of Right Lamina at junction with Transverse process (Mean ±SD)</th>
<th>Height of Right Lamina at junction with Spinous process (Mean ±SD)</th>
<th>Height of Left Lamina at junction with Transverse process (Mean ±SD)</th>
<th>Height of Left Lamina at junction with Spinous Process (Mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>24.65 ± 0.63</td>
<td>26.45 ± 0.54</td>
<td>24.65 ± 0.63</td>
<td>26.45 ± 0.54</td>
</tr>
<tr>
<td>L2</td>
<td>25.7 ± 0.61</td>
<td>27.5 ± 0.74</td>
<td>25.7 ± 0.61</td>
<td>27.5 ± 0.74</td>
</tr>
<tr>
<td>L3</td>
<td>25.4 ± 0.68</td>
<td>25.9 ± 0.62</td>
<td>25.4 ± 0.68</td>
<td>25.9 ± 0.62</td>
</tr>
<tr>
<td>L4</td>
<td>22.35 ± 0.66</td>
<td>23.4 ± 0.67</td>
<td>22.35 ± 0.66</td>
<td>23.4 ± 0.67</td>
</tr>
<tr>
<td>L5</td>
<td>20.75 ± 0.44</td>
<td>21.25 ± 0.59</td>
<td>20.75 ± 0.44</td>
<td>21.25 ± 0.59</td>
</tr>
</tbody>
</table>
### Table 2: Comparison of the Height of lumbar vertebrae lamina bilaterally between Male and Female.

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Height of Right Lamina at junction with Transverse process in mm (Mean ±SD)</th>
<th>Height of Right Lamina at junction with Spinous process in mm (Mean ±SD)</th>
<th>Height of Left Lamina at junction with Transverse process in mm (Mean ±SD)</th>
<th>Height of Left Lamina at junction with Spinous Process in mm (Mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (10)</td>
<td>Female (10)</td>
<td>Male (10)</td>
<td>Female (10)</td>
</tr>
<tr>
<td>L1</td>
<td>24.4 ± 2.5</td>
<td>24.9 ± 3.2</td>
<td>26.9 ± 2.5</td>
<td>26 ± 2.4</td>
</tr>
<tr>
<td>L2</td>
<td>25.6 ± 2.5</td>
<td>25.8 ± 3.1</td>
<td>28.7 ± 3.6</td>
<td>26.4 ± 2.7</td>
</tr>
<tr>
<td>L3</td>
<td>25.1 ± 2.1</td>
<td>25.7 ± 3.83</td>
<td>25.9 ± 2.64</td>
<td>26 ± 3.09</td>
</tr>
<tr>
<td>L4</td>
<td>22.5 ± 2.6</td>
<td>22.2 ± 3.42</td>
<td>23.7 ± 3.05</td>
<td>23.1 ± 3.14</td>
</tr>
<tr>
<td>L5</td>
<td>21 ± 1.63</td>
<td>20.5 ± 2.32</td>
<td>21.7 ± 2.83</td>
<td>20.8 ± 2.52</td>
</tr>
</tbody>
</table>

### Table 3: Transverse width of Vertebral canal.

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Transverse width of vertebral canal (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>14-23</td>
</tr>
<tr>
<td>L2</td>
<td>14 - 23</td>
</tr>
<tr>
<td>L3</td>
<td>13 - 22</td>
</tr>
<tr>
<td>L4</td>
<td>12 - 21</td>
</tr>
<tr>
<td>L5</td>
<td>12 - 21</td>
</tr>
</tbody>
</table>
Discussion

Clinical significance of morphometric study of Lumbar Canal and Lamina is a well-established fact.

Standard Laminectomy surgery is routinely performed to relieve the symptoms of nerve compression in lumbar canal stenosis. It is also done for lumbar disc herniation. The use of intralaminar screw and laminar hooks is also gaining popularity. Laminar screws are used for spine fixation during fractures and in scoliosis correction. The knowledge of morphometry of lumbar lamina is thus of paramount importance for spinal surgeons for performance of laminectomy & successful placement of implants locally.

The present study has generated a data base for cadaveric study of gender-wise dimensions for lumbar vertebrae lamina. The present study has measured the
different dimensions of lumbar vertebrae in cadavers with the use of digital vernier caliper. Azu et al (2016) in their study have asserted that direct measurements may give more accurate and reproducible results compared to those from imaging technique10. Tharani et al (2018) have studied the height of lumbar vertebra lamina on dry vertebra14. The mean height of lamina in their study was found to be smaller than that of present study bilaterally. However, in their study also the mean height of L2 lamina was found to be largest and showed gradual decrease from L3-L5. This finding is analogues to the present study. The research has established that the anatomical narrowing of the lumbar vertebral canal and intervertebral foramina may cause compression of the cauda equina and the emerging nerve roots. The manifestations of compression are pain in the lower back and lower limbs on walking, weakness and paraesthesia along the distribution of the affected nerve roots.

Transverse diameter of the lumbar vertebral canal (interpedicular distance) is a reliable index for the assessment of the size of the canal2. So, the present study has attempted to determine the data base for transverse diameter of lumbar vertebral canal. There was no significant difference in width of vertebral canal between Male and female at any level.

The result of present study for transverse width of vertebral canal varies from the previous reports in the literature. Tarek et al (2013) in their study have reported that the mean transverse width gradually increases from L1 to L5. He has reported that the transverse diameter in lumbar vertebral canal are 1 to 1.5 mm higher in males while in present study the it was found to be higher in females5.

**Conclusion**

The present study has determined the dimensions of lumbar vertebral canal & lamiae in Indian population. The data so collected will be of great significance for spinal surgeons dealing with this region and also for designing of implant used around this region.

**Acknowledgement:** I express my sincere gratitude to the generous donors who donated their bodies for academic and research purposes. Without cadavers this research would have not been possible.

**Source of Funding:** No funding.

**Conflicts of Interest:** The author declares no conflict of interest.

**Ethical Clearance:** Ethical approval to undertake the present study was obtained from the Institution Ethical Committee (IEC)

**References**


Screening for Lower Cross Syndrome in Asymptomatic Individuals- A Study Protocol

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Abstract

Background: lower cross syndrome results from muscle strength imbalance in the lower segment. It is characterized by specific pattern of muscle weakness and tightness that cross between the dorsal and ventral sides of the body. In lower-crossed syndrome, the patient typically has anterior pelvic turn, elevated lumbar lordosis (swayback), and weak abdominal muscles. Inaction may also have a detrimental effect on the mechanics of the body, such as immobilization, disuse or excessive postural discomfort, such as sitting at the workstation for long periods of time and poor posture .It also involves tightness of the iliopsoas, rectus femoris, fascia lata tensor, adductor group, gastrocnemius, and soleus. Pressure biofeedback device is used for the purpose of measuring and treating the transverse abdominis muscle. This study is needed because over time due to prolonged daily activities and lack of regular exercises in young people, this leads to muscle imbalance which leads to low back pain in this population. The goal of the study is to screen for lower cross syndrome in asymptomatic individual.

Aim and Objective: To screen for lower cross syndrome in asymptomatic individual.

Materials and Methods: Based on the inclusion and exclusion criteria, 300 individuals will be included in the study and the respective evaluation procedure for lower cross syndrome will be carried out.

Result: The expected results would include the data about whether lower cross syndrome would be detected in the asymptomatic individuals. Data will be analyzed using paired T-test.

Conclusion: based on the previous data of test and assessment we assume it will be beneficial in relieving the symptoms of lower cross syndrome.

Keywords: lower cross syndrome, Kinect azure, pressure biofeedback unit, Thomas test.

Introduction

Lower Crossed Syndrome (LCS) is a musculoskeletal condition characterized by unique patterns of muscle weakness (Abdominals and Gluteus Maximus) and tightness (iliopsoas and spinal extensors) that connect the dorsal and ventral sides of the body. This postural difference will lead to future Low back pain. This research therefore attempts to establish the screening of lower crossed syndrome in young people.

Lower cross syndrome is also known as pelvic cross syndrome or underkruz syndrome1, resulting in a disparity of muscle strength between deep abdominal musculature and gluteus medius , and gluteus maximus. This is a syndrome of postural distortion which affects the lower kinetic chain. The patient typically has anterior

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pelvic turn, elevated lumbar lordosis (swayback), and poor abdominal muscles in lower-crossed syndrome. These patients usually suffer from severe low back pain, piriformis syndrome and anterior pain in the knees. The lower cross syndrome involves oblique rectus abdominis, gluteus maximus, medium gluteus and hamstring. It also involves iliopsoas tightness, rectus femoris, tensor fascia lata, adductor group, gastrocnemius, and soleus. It results in anterior pelvic movement, lumbar spine hyperlordosis, flexion hips and hyperextended knees. From a variety of scenarios such as chronic, repeated, running acts, low-cross syndrome can develop. Inaction may also negatively impact the body’s mechanics, such as immobilization, disuse or constant postural pain, such as sitting for long periods of time and bad posture at the workstation. Lower cross syndrome is described in two distinct ways: one in the lower back and one in the hip. Rehabilitation of the lower cross syndrome is based on the Sherrington law of reciprocal inhibition which states that the opposite muscle relaxes when one muscle is shortened or tightened. So the pelvis, hip joint and lumbar spine will relax, with the stretching of compressed muscles and muscle strength. Some muscles are overactive while others are inhibited as the muscles respond to protect the body from damage or pain.

As a result, joint stress is altered and muscle tiredness is increased. Postural or anti-gravity muscles are more readily activated for these defensive reactions. Meanwhile, muscles with predominantly dynamic or physical function appear to be impaired when physical stress occurs. When muscles react to prevent or reduce physical injury, normal patterns of movement are changed. Muscle strength affects performance of released motion. Additional effects of muscle imbalance include altered joint mechanics that cause unequal distribution of articular pressure and altered rotational centers that eventually contribute to joint instability and pain. The combination of strong and weak muscles induces changes in movement patterns resulting in changes in joint biomechanics leading to secondary degenerative changes. With a limited range of joint motion due to chronic tightness of the muscle, secondary associated joint hypermobility problems may occur.

Pressure biofeedback unit is used for the evaluation and treatment purpose of the transverse abdominis muscle. If the lordosis is deep and small, then the imbalance is mainly in the pelvic muscles; if the lordosis is shallow and extends into the thoracic region, then the imbalance predominates in the muscles of the trunk. Janda considered muscle imbalance to be an impaired relationship between muscles prone to tightness or shortness, and muscles prone to inhibition. According to the source, our body primarily has two types of muscles, which are postural muscles such as iliopsoas, lumborum quadrates, erector spinae, back rotators, and phasic muscles such as abdominals, hip extensors, etc. He noted that static or postural muscles are predominantly tending to tighten. Individuals in the age group from late adolescence to 40 years are extremely involved in everyday life and are subject to different stresses.

In contrast to older people, the age-related changes in this age group are minimal. One of the most interesting hypotheses in physical medicine is that the fundamental mismatch between postural and phasic muscles in and around the pelvis perpetuates tension, strain and altered patterns of movement that account for the re-injury of these main structures.

Janda noticed that the hip flexors become shortened or tight due to prolonged static postures, such as sitting at a desk all day. The brain will therefore automatically start shutting down or inhibiting the muscles of the glutei that are on the opposite side. Now, due to the forward pelvic turn and hip flexion contracture, the imbalance pattern facilitates increased lumbar lordosis and over activation of hip flexors compensating for the weak abdominals.

Hip extension would be limited due to tightened psoas, and the presence of an inhibited antagonistic gluteus maximus would create a poor quality of hip extension because the contra-lateral lumbar erector spinae and ipsilateral hamstrings would have to become overactive and eventually tight to perform the necessary hip extension instead of the gluteus maximus. Low back pain is a common problem and lower crossed syndrome is one of the threatening combinations of lower back structures with biomechanical muscle dysfunction due to excessive stress. People with such postural imbalance
sometimes complain of lower back pain and, if left unchecked, this postural imbalance may trigger a chronic lower back pain that becomes more difficult to correct at later stage.

Das S, Sarkar B, et al. This study showed that the prevalence of lower crossed syndrome was higher in females than in males of the same age group. It was also found that there is insignificant difference between males and females of the same age group in iliopsoas (left sided) tightness. But there is a significant difference in length of spinal extensor, abdominal strength and maximum strength of bilateral gluteus, right iliopsoas length between males and females of the same age group, and females are more prevalent in developing weakness of these muscles. However in this study, the prevalence was not significant to the number of individuals screened.¹

There is need for this study because over the period of time due to prolonged daily activities and lack of regular exercises in young individuals, leads to muscle imbalance leading to low back pain in this population. Therefore the awareness regarding regular exercise and stretching would be possible by, screening individuals for lower cross syndrome flags in asymptomatic individuals for early detection, treatment, and prevention.

**Objective:** To screen for lower cross syndrome in asymptomatic individual and to prevent lower cross syndrome in the young individuals.

**Methods**

This research is being conducted in local communities in Wardha City, Maharashtra, India after ethical clearance is obtained from Institutional Ethics Committee, Datta Meghe Institute of Medical Sciences, Deemed to be University. Based on the inclusion and exclusion criteria, 300 individuals will be included in the study and the following evaluation procedure will be carried out.

**Study Design:** Observational study

**Settings:** Datta Meghe Institute of Medical Science, Sawangi Meghe, Wardha

**Participants:**

Inclusion criteria:

1. Asymptomatic individual,
2. Patients willing to participate

Exclusion criteria:

1. Chronic low back pain
2. Disc herniation,
3. Neurological deficits,
4. Respiratory conditions,
5. Patients who are not willing to participate or uncooperative.

**VARIABLES:**

Outcome measures:

There will be assessing of the following muscles:

1. **Postural assessment**- The posture will be evaluated with Kinect Azure which will provide detailed evaluation of posture in anterior, posterior and lateral view.³ Typically, Kinect data showed excellent validity and could be used to predict dynamic foot work. This approach has also been used for other anatomical measurements, including calculation of the curvature of the spinal cord, patient size, and lung function of the respiratory volume.

2. **Thomas Test**- This test is used to measure hip flexor muscle flexibility, which includes iliopsoas, rectus femoris, pectineus, gracilise, tensor fascia late and sartorius. The patient is normally supine, investigate excessive lordosis. Flex one hip, bring the knee to the chest to flatten the spine / support the pelvis. The patient keeps a flexed hip to the chest. If anterior tightness leg is present, the leg will lift off the bed. Intrarater reliability =0.52 interrater reliability =0.60, SEM =1 degree.⁴

3. **Gluteus Maximus Strength Test**: The patient’s position is inclined to lie with one knee flexed up to 90 degrees. The patient lifts the thigh from the table while the examiner pushes the thigh raised towards the table. Compare bilateral muscle strength. The ICCS Ranged from 0.76-0.85 and from 0.75-0.83.⁵

4. **Psoas Major Strength Test**- The patient position is supine with one leg elevated, abducted and
foot rotated externally 45 degrees. The patient elevates the leg and examiner resists it and stabilises the opposite ASIS. Compare the muscle strength bilaterally. It has a inter examiner reliability of cohane k unweighted score (κ- 0.67).  

5. **Modified Schobers test:** It measures the amount of flexion in the lumbar. The position of the patient is on his back while the examiner is standing. A mark is made on the vertebral column at the level of PSIS. Mark 5 cm below it and 10 cm above it, then ask to touch the toes. Unless the rise in distance between the two fingers on the spine of the patient is less than 5 cm then this is indicative of restricting lumbar flexion or overly active erector spinae. The high values of Intraclass Correlation Coefficients (0.83 for flexion and 0.68 for extension) also indicated high reliability for between-days measurements.

6. **Modified Thomas test:** this test is for rectus femoris muscle Patient posture is likely to lie with knees bent to 90 degrees, the examiner asks patients to resist while pushing against the flexed knee. Compare the strength of the muscles bilateral. it is having interrater reliability (r =0.91-0.93; ICC= 0.89-0.92) 

7. **Transverse Abdominal Muscle**- The patient position is prone lying asks to pull in the stomach by increasing abdominal pressure stabilising the lumbar spine.

8. **Active knee extension test:** It is used to measure the length of the hamstring muscle and the extent of active knee extension in the hip flexion role. The patient lies supine with and is advised to move his leg to full hip flexion with arms raising his forearm to the chest; the patient is advised to vigorously flex the knee from the starting position to the full tolerable stretch in the hamstring muscle. Measure the angle with the goniometer. This test has excellent interrater reliability with interclass correlation coefficient (ICC) values of 0.87 and 0.81, standard error of measurement (SEM) = 3.5 and 3.8 degrees.

9. **Pressure biofeedback unit:** A system used to test transversus abdominis. It is a simple instrument that records varying pressure in an air-filled pressure cell. This allows identification of body along with the spinal movements. This system functions as a stabiliser and can be used for activities focusing on joint protection and stabilization. This study found 0.47 [95 percent confidence interval (CI) 0.20 to 0.67] intraclass correlation coefficient (ICC) for inter-observer reliability, and 0.81 (95 percent CI 0.67 to 0.90) ICC for test-retest reliability.

**Study size:** 300

**Discussion**

Muscle dysfunction is one of the most significant risk factor of low back pain in the young population. Lower Crossed Syndrome (LCS) is a muscle imbalance that includes tightness of hip flexors, lower back muscles and abdominal weakness, gluteus maximus muscle; that alters the distribution of biomechanical force in lower back region and can lead to chronic low back pain. There is lack of literature which states the screening of lower cross syndrome in asymptomatic individuals. The present study will be conducted with the aim of screening the asymptomatic individuals suffering from lower cross syndrome.

In the pelvic cross syndrome originally suggested by Janda, the pelvis is more dorsal and this is associated with imbalanced trunk muscle coactivation with more dominant activity found in the extensors termed as posterior pelvic cross syndrome. Conversely, pelvis is more anterior in the other large category and this is associated with a predominant tendency for more flexor activity, known as anterior pelvic cross syndrome.  

**Expected Results**

Lower cross syndrome was detected in the young individuals but no study has been done on asymptomatic individuals. The result would include data about whether the measures taken were effective for screening lower cross syndrome. Once the study result is complete, data will be analysed using paired t-test and will be presented in the form of research paper.

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Unnatural Deaths in the Paediatric Age Group in a Tertiary Hospital at Bangalore: An Autopsy Study

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Abstract

Background and Aim: During transition from childhood to adulthood, people are exposed to various hazards having potentiality lead to unnatural deaths by distorting physical, mental and social wellbeing. Studying pattern of unnatural deaths helps stakeholders to formulate policies for prevention of loss of important human resource.

Materials and Methods: A descriptive study with purposive sampling was conducted at KIMS Hospital and Research Centre, Bangalore from 2013 to 2015 wherein the data using pre-tested structured proforma was collected from autopsies of unnatural deaths among pediatric age group (0-18 years). The details pertaining to the Socio demographic characteristics, pattern of injuries and cause of death was ascertained from the police reports, reliable attendants of the deceased, histopathological /hospital records and postmortem reports. The data obtained from this study was analyzed statistically by presenting the data in the form of appropriate tables and graphs, computing the descriptive statistics like mean, median, standard deviation and percentages.

Results: Out of 832 cases, 703 (84.5%) were unnatural deaths, amongst which 8.8% cases were of paediatrics of age group. In that (82.2%) 51 cases belonged to 12-18 years, followed by 5-12 years and preschool age (3-5years) each with 4 cases (6.5%), 3 cases (4.8%) are of toddlers (1-3) years and no cases were reported among infants. Male preponderance was seen 35 cases (56%). Maximum number of deaths occurred during evening times i.e., 30 cases (48.39%). In the present study, maximum number of victims were from social class II i.e., 38 cases and least among social class I with only 1 case. Out of 62 cases , 37 cases (60%) were intentional deaths (1 case (3%) is homicidal and 36 cases (97%) were suicidal) and 25 cases (40%) were unintentional deaths. In the present study out of 36 suicidal cases, Hanging was the most preferred method used to commit suicide with 26 cases (42%), followed by burns and drowning with 1 (8%) case each. Out 62 cases, only 12 (19%) cases were treated before death and remaining 50 (81%) were not treated prior to death.

Conclusion: This study indicates that there is an immense responsibility for the parents and caregivers in supervising their children. The public health burden of all unnatural deaths needs to be estimated to provide a rational basis for policy decisions to implement necessary interventions.

Key Words: Unnatural deaths, Paediatric age group (0-18years), Suicide.

Introduction

It’s been aptly said that “Child is the father of Man” and indeed the health status of a community is reflected by the pediatric age group (0 to 18 years) in a given area. It is estimated that all over the world, more than 26,000 children under the age of five mostly from developing
countries die every day.\(^1\) India contributes one-sixth of the world’s population, 29.5% of which belongs to the 0–14 year age group.\(^2\)

A child of today is subjected to so much of stress starting from strained relationship between parents resulting in broken homes, expectations to perform better at the academic arena, comparison between children, sense of self neglect etc. The pattern of deaths among paediatric age group has changed from infections towards social etiologies during the last decades. Injury and violence are major killers of children because of the effects on social, physiological, economic and medical issues throughout the world, responsible for deaths in children and young people under the age of 18 years each year, causing public health issues.\(^3\)

Keeping this in mind, a study was undertaken with the objectives to describe the demographic profile, to assess the proportion and causative factors of unnatural deaths in paediatric age group. The rationale of determining the features and pattern of medico legal deaths in childhood will contribute to the establishment of a database for the formulation of national and international policies helping to lower the mortality rate in the paediatric age group.

Methodology

Using pre-tested structured proforma, data were collected from all cases of unnatural deaths among paediatric age group (0-18 years) brought to KIMS Hospital and Research Centre, Bangalore for a period of 18 months for autopsy. The details pertaining to the demographic characteristics, manner, time and place of death were ascertained from the investigating police officers, police reports and reliable attendants of the deceased. The data obtained from this study was analyzed statistically by presenting the data in the form of appropriate tables and graphs, computing the descriptive statistics like mean, median, standard deviation and percentages.

Results

During the period (November 2013 to April 2015), 832 cases were brought for post mortem examination; out of which 703 (84.5%) deaths were due unnatural causes. Out of 703 cases of unnatural deaths, 62 (8.8%) deaths were of 0-18 years age group. Out of 62 cases, Male:Female ratio was found to be 1.29:1. Age wise distribution showed that 3 cases (4.8%) belonged to 1-3 years (Toddler), 4 (6.5%) cases were of the age group 3-5 years (Pre-school), 4 (6.5%) were of 5-12 years (School age) and 51 (82.2%) were of 12-18 years (teen). (Fig 1)

The maximum number of unnatural deaths occurred during evening times i.e. 30 cases (48.39%) and the least number of cases were recorded between 12 am to 6AM (3 cases, 4.84%). (Table 1)

As per the Modified Kuppuswamy scale (update for February 2019), this study of 62 cases had maximum victims from social class II i.e., 38 (61%) cases and least among social class I i.e., 1 case which accounted for 2%. (Fig – 2)

Amongst the 62 unnatural deaths in paediatrics, 37 (60%) cases were intentional and 25 (40%) cases were unintentional. 1 case of intentional death (3%) was homicidal and 36 cases (97%) were suicidal in nature. Homicidal deaths was of a male victim, while in case of suicidal deaths 14 victims were males and 22 were females.

Hanging was the most common manner of death with 26 cases (42%) (12 cases males and 14 cases of females) out of 62 cases, followed by 10 cases (15%) of R.T.A (8 cases of males and 2 cases of females) and the least common were seen in electrocution and strangulation, accounting to 1 male victim case each (2%). (Table 2)

Out of 36 suicidal cases, Hanging was found to be the most preferred method used (72%), followed by poisoning (22%), burns and drowning accounting to 3% of cases each. Depression followed by failure in studies, family problems, love failure and ill health issues were found to be the reason for committing suicide. 1/4th of them had previously attempted suicide.

Out of 25 cases of unintentional deaths, 40% cases were due to road traffic accidents followed by drowning and fall from height (24% each) with the least being due to electrical shock (4%). Out of 62 cases, more than 3/4th of them did not receive treatment prior to death (Table 1)
Fig 1: AGE INCIDENCE OF THE VICTIMS (0-18 years):

Fig 2: SOCIOECONOMIC STATUS OF THE VICTIMS
### TABLE-1: Profile of Paediatric Victims of Unnatural death (n=62)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of Cases</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIME</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:01am to 6:00am</td>
<td>3</td>
<td>4.84%</td>
</tr>
<tr>
<td>6:01am to 12:00pm</td>
<td>18</td>
<td>29.03%</td>
</tr>
<tr>
<td>12:01pm to 6:00pm</td>
<td>11</td>
<td>17.74%</td>
</tr>
<tr>
<td>6:01pm to 12:00am</td>
<td>30</td>
<td>48.39%</td>
</tr>
<tr>
<td><strong>INTENTIONAL DEATHS (n=37)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homicides</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Suicides</td>
<td>36</td>
<td>97%</td>
</tr>
<tr>
<td><strong>HISTORY OF PREVIOUS SUICIDAL ATTEMPTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>25%</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>75%</td>
</tr>
<tr>
<td><strong>METHODS EMPLOYED TO COMMIT SUICIDE (n=36)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hanging</td>
<td>26</td>
<td>72%</td>
</tr>
<tr>
<td>Poisoning</td>
<td>8</td>
<td>22%</td>
</tr>
<tr>
<td>Burns</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Drowning</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td><strong>TYPE OF UNINTENTIONAL DEATHS (n=25)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.T.A</td>
<td>10</td>
<td>40%</td>
</tr>
<tr>
<td>Drowning</td>
<td>6</td>
<td>24%</td>
</tr>
<tr>
<td>Electrical shock</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Fall from height</td>
<td>6</td>
<td>24%</td>
</tr>
<tr>
<td>Burns</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td><strong>INCIDENCE OF ROAD TRAFFIC ACCIDENTS AND ITS SEX DISTRIBUTION (n=10)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>80%</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>20%</td>
</tr>
<tr>
<td><strong>TREATMENT PRIOR TO DEATH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>12</td>
<td>19%</td>
</tr>
<tr>
<td>Not Treated</td>
<td>50</td>
<td>81%</td>
</tr>
</tbody>
</table>
TABLE: 02: MANNER OF DEATH WITH SEXWISE DISTRIBUTION

<table>
<thead>
<tr>
<th>Manner of Death</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td>Hanging</td>
<td>12</td>
<td>46%</td>
<td>14</td>
<td>54%</td>
<td>26</td>
<td>42%</td>
</tr>
<tr>
<td>Poisoning</td>
<td>2</td>
<td>25%</td>
<td>6</td>
<td>75%</td>
<td>8</td>
<td>13%</td>
</tr>
<tr>
<td>Burns</td>
<td>1</td>
<td>33%</td>
<td>2</td>
<td>67%</td>
<td>3</td>
<td>5%</td>
</tr>
<tr>
<td>Fall from height</td>
<td>5</td>
<td>83%</td>
<td>1</td>
<td>17%</td>
<td>6</td>
<td>10%</td>
</tr>
<tr>
<td>Drowning</td>
<td>5</td>
<td>72%</td>
<td>2</td>
<td>28%</td>
<td>7</td>
<td>11%</td>
</tr>
<tr>
<td>R.T.A</td>
<td>8</td>
<td>80%</td>
<td>2</td>
<td>20%</td>
<td>10</td>
<td>15%</td>
</tr>
<tr>
<td>Electrocution</td>
<td>1</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>Strangulation</td>
<td>1</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>2%</td>
</tr>
</tbody>
</table>

**Discussion**

During the 18 months of study, only 62 cases of unnatural deaths among paediatric age group 0-18 years were studied. It represents only a part of problem of unnatural deaths among paediatric age group in Bengaluru as such cases are distributed in different mortuaries catering to different regions/areas of Bengaluru.

Among 62 cases of unnatural deaths in paediatric age group more deaths were seen in the age group of 12-18 years (Teens) i.e. 51 (82.2%) cases. The mean age of the males was 16.0 years while that of the females was 14.8 years in Ohene’s and Meel’s report. Unnatural deaths increase with age, since there is more violence expected in older children. This could be attributed to mental instability and hormonal influence. Proper parental guidance and suitable moral support should be provided for the same.

35 (48.2%) cases were males and 27 (51.8%) cases were females. The male-to-female ratio was 2.1:1, as recorded in Sally’s report. This might be because males are more prone active, curious and adventurous by nature.

Majority of unnatural deaths in the study occurred during evening (48.39%) and mid morning (29.03%). There must be an immense responsibility for the parents and caregivers in supervising this age group. Most of the victims were from Middle class in 85% of cases and least among Upper class i.e. 2%. An administrative policy of the country thereby plays a vital role in mortality and morbidity rates. Financial security of the weaker sections should be enhanced. 60% (37 cases) of the cases were intentional and 40% (25 cases) were unintentional deaths. Among unintentional deaths, 97% (36 cases) were suicidal and 3% (1 case) was homicidal in nature. Suicide in the past decade is an increasing trend with the increasing population of India, according to the National Crime Records Bureau. According to recent study, the suicide rate per 100,000 population among females of 10-19 years was 11.98, while it was 11.9 per 100,000 population among males of the same age in Sri Lanka by 2011. Suicides tended to be more prevalent among older teenagers. At that stage of life, teenagers...
feel distressed about their grades, sex, future plans, and jobs. Children less than 14 years of age less frequently suffer from psychiatric disorders, family conflicts, or romantic disappointment. Moreover, children do not develop insight in the definitiveness of death before the age of 12 or 14 years.

Hanging was the most common method of suicide in Canada for child victims of suicide between the ages of 10-19 years. In the present study the most common reason for committing suicide is depression in 53% of cases. As reported in literature, psychological autopsy has a paramount importance on elucidating death investigation on children and adolescent suicide, regarding the analysis of the implied risk factors. In these cases, psychological autopsy was fundamental to understand the suicidal behaviour and to determine the manner of death. From the present study among unintentional deaths R.T.A is the most common cause accounting for 40% followed by Drowning and Fall from Height with 24% each. Traffic crashes are the leading health threat to children in many countries.

This may be due to the increased involvement of males in outdoor business and increased usage of vehicles by males. Alcohol abuse may be another factor which could be associated.

Conclusion

Unnatural dying is abrupt, and traumatic. Violence, Violation and Volition are its unique dimensions. Unnatural deaths among paediatrics are predictable and preventable. Education and employing effective strategies has a role to play in prevention, especially when it is paired with technology and legislation. If not preventable, prompt and immediate care should be provided in order to save the life of the victim.

Ethical Clearance: Obtained from IRB committee

Conflict of Interest : Nil

Source of Funding: Self

References

Estimation of Time Since Death from Na\(^+\) Ion Concentration in CSF

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Abstract

In the present study 100 medico legal cases examined for Na\(^+\) ion concentration in CSF (cerebrospinal fluid) at different postmortem interval in the department of Forensic Medicine Indira Gandhi Medical College, Nagpur. Analysis revealed that there is decrease in sodium ion concentration with PMI, and shows negative coefficient correlation r= -0.721. (p<0.001), the least square regression equation is found to be \(y = -1.027x + 138.7\), where \(y\)= sodium ion concentration (independent variable) and \(x\)= time since death (dependent variable). The sodium ion concentration in CSF decreases after death and it is statistically significant but individual variation is such that we can’t accurately calculate postmortem interval from sodium ion concentration.

Key Words: Na\(^+\) ion concentration, time since death, postmortem interval (PMI)

Introduction

Time since death that is the interval between death and the time of post-mortem examination also called as “Post-mortem interval”. It provides a clue to the investigating officer to institute suitable enquiries to apprehend the persons likely to be responsible for the crime and to eliminate the innocent ones.\(^1\) As per earlier study it was concluded that the sodium in CSF decreases with increasing post-mortem interval and decrease is not significant and hence cannot be utilize precisely to estimate post-mortem interval.\(^6\)

Therefore an attempt has been made with optimistic hope and expectation by carrying out a study of estimation of time since death from Na\(^+\) ion concentration in CSF

Material and Methods

100 medico legal autopsies were selected where the exact time of death was known and the body had been kept in prevailing room temperature.

Inclusion criteria

1) Only those cases are included in the study whose time of death is known.

Exclusion criteria

1) Any cases of head injury, brain pathology, bleeding diathesis were excluded.

2) Decomposed bodies were excluded.

3) Cases of diabetes and if during postmortem examination kidney and liver shows gross pathological abnormality those cases were excluded.

The cranial cavity was opened in usual way and the vault will be removed to expose the brain and dura.
Dura is gently cut from the vault region of the brain both cerebral hemispheres are separated apart from midline by left hand to expose corpus callosum 10ml plastic disposable syringe with wide bore canula (18 gauge) is inserted on the posterior and depended part of corpus callosum for 1.5cm depth. The needle is directed posteriorly, downwards and slightly laterally in each hemisphere and cerebrospinal fluid was withdrawn and immediately centrifuged and analyzed for sodium ion on Dimension Xpand plus automatic analyzer (indirect ion selective method) after centrifugation, in the dept. of biochemistry IGGMC Nagpur.

Observation and Results

In the present study out of 100 cases 68 cases were males 32 cases were females, 37 cases were studied in summer, 38 cases were studied in rainy days, 25 cases were studied in winter. Environmental temperature ranges from 30 - 46°C in summer, 22 – 39°C in rainy days and 18 – 33°C in winter. Cause of death was poisoning in 24 cases, blunt trauma to chest in 11 cases, coronary artery disease in 16 cases, burns in 13 cases, hanging in 9 cases, snake bite in 1 case, septicaemia in 10 cases, drowning in 2 cases, peritonitis in 3 cases and 3 were bilateral pulmonary consolidation cases. The cases were grouped into four groups on the basis of postmortem interval (PMI) i.e. 0-6, >6-12, >12-18, >18-25. The list PMI observed was 2 hours and maximum was 25 hours.

The below table and graph is showing decrease in sodium ion concentration with PMI, and shows negative coefficient correlation $r=-0.721$. ($p<0.001$).

The mean value is 127.37mEq/L and value ranges from 103mEq/L to 144mEq/L with a standard deviation ±9.48 mEq/L. The sodium ion values showed a significant negative correlation coefficient $r = -0.721$ (i.e. Na$^+$ values decrease with time since death) which is statistically significant. ($p<0.001$)

The least square regression equation is found to be $y = -1.027x + 138.7$, where $y =$ sodium ion concentration (independent variable) and $x =$ time since death (dependent variable)
Table showing distribution of cases in different postmortem interval, with mean values and standard deviation, and range of values of Na⁺ ion in that postmortem interval

<table>
<thead>
<tr>
<th>PMI Group</th>
<th>0-6</th>
<th>&gt;6-12</th>
<th>&gt;12-18</th>
<th>&gt;18-25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±S.D.</td>
<td>134.32±7.89</td>
<td>130.71±5.66</td>
<td>123.35±8.082</td>
<td>117.71±4.34</td>
</tr>
<tr>
<td>Values of Na⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranges From</td>
<td>144 to 110 mEq/L</td>
<td>140 to 119 mEq/L</td>
<td>137 to 107 mEq/L</td>
<td>126 to 103 mEq/L</td>
</tr>
</tbody>
</table>

The 95% limits of confidence interval of CSF sodium ion, at 0-6 hour postmortem interval are 134.32±2.75 mEq/L, at >6-12 postmortem interval are 130.71±2.58 mEq/L, at >12-18 hour postmortem interval are 123.35±3.49 mEq/L, at >18-25 hour postmortem interval are 117.39±1.83 mEq/L.

**Discussion**

Nauman H.N. (1958) had analyzed 157 samples of postmortem CSF consisting of 131 males autopsied on the average 10.5 hours after death. He found that the postmortem CSF sodium ranges from 107 to 150 meq/L, with an average of 127 meq/L.²

Paulson G.W. and Stickney D. (1971) evaluated the postmortem CSF for sodium. In his 17 cases studied from postmortem interval of 1.5 hour to 17 hour, the value at six hour is 120 and 149 meq/L (2 cases); at 11 to 14 hours, the value was 138 and 129 meq/L (2 cases); and at 17 hour, it was 130 meq/L (1 case). He noted that there was fall of sodium in the postmortem period.³

Karkela J.T. (1993) studies the sodium in the postmortem CSF of 40 different adult cadavers. The samples were drawn at 2, 4, 10 and 24 hour after death and found mean values for Na⁺ 141 mmol/L at 2 hour, 139 mmol/L at 4 hour, 131 mmol/L at 10 hour and 122 at 24 hour.⁴

Bardale R. V. and Dongre A.P. (2004) studied CSF in 100 medicolegal cases. They noted that there was decrease of sodium concentration with increasing postmortem interval, at sixth hour of PMI, the values ranges between 88.3 to 210 meq/L with an average of 124.8 meq/L and SD 20.01. There was decrease of sodium at 12 hour PMI with range of 56.4 to 175.4 meq/L with a mean of 103.46 meq/L, and SD 18.12. By 18 hour after death, the value of sodium ranges from 42.1 to 152 meq/L with a mean of 85.60 meq/L, and SD 18.73.⁵

Yadav J et al (2007) Results revealed a significant correlation of sodium and potassium ion in CSF up to 25 hours of time since death. The study concludes that changes in CSF electrolytes are a significant parameter to estimate time since death.⁶

We found the mean value is 127.37 mEq/L and value ranges from 103 mEq/L to 144 mEq/L with a standard deviation ±9.48 mEq/L. The sodium values showed a significant negative correlation coefficient r = - 0.721 (i.e. Na⁺ values decrease with time since death) which is statistically significant. (p<0.001)

My findings are consistent with Yadav J et al, Bardale R. V and Dongre A.P., Karkela J.T., Paulson G.W. and Stickney D. and Nauman H.N.

**Conclusion**

The sodium ion concentration in CSF decreases after death and it is statistically significant but individual variation is such that we can’t calculate postmortem interval from sodium ion concentration.

**Acknowledgement :** None
Conflict of Interest – no conflict of interest

Source of support – not applicable

Ethical Clearance – institutional ethical committee clearance taken

References


Effect of Vachadi Ointment in the Management of Scabies - A Case Report

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Abstract

Introduction: Skin disease has a high prevalence throughout the world. Scabies is contagious and can spread quickly through close physical contact so it is an acute communicable disease. It is highly prevalent in young children. The present study is aimed to show the effect of Vachadi ointment on scabies in children. Scabies is characterized by nocturnal itching, vesicular or pustule eruptions, with small red bumps and blisters. According to Ayurveda Scabies can be correlated to Pama. Pama is one of the eighteen types of Kushtha (Skin Disease) according to the Charak Samhita (Chikitsa-Sthana) which is caused by vitiation of Kapha and Pitta Dosha. Case Description: A 10 years old male patient brought with complaints of itching in between the fingers of both the hands, wrists and forearms since 10-12 days. On local examination, vesiculopustular lesions were present along with dry scaly patches over both the hands, wrists and forearms. Effect of the treatment was assessed on the basis of clearance of lesion and relief from itching. Conclusion: After intervention of the Vachadi ointment for 14th day, there was a marked improvement in symptoms like itching, scaling, and discharge. So, it can be said that Ayurveda has a better remedy for skin related complaints.

Key words:- Pama, Scabies, Communicable, Vachadi, Kushtha

Introduction

Skin is largest and most important organ of the body as it protects the internal organs from the harmful environmental influences. Many agents can affect the skin they may be varying in nature - physical (trauma, heat), chemical (strong acids) and biological (variety of organisms). The skin has several inbuilt mechanisms for interacting with the environmental agents and most of the times the skin is able to protect it from those agents. The protective ability however, may not always be able to deal with the environmental stimulus and this leads to the production of skin disease.¹ Skin diseases in Ayurveda are described as Kushtha roga. There are seven Mahakushta and eleven number of Shudra Kushtha roga are explained.² All Kushtha Roga are Tridoshaja in nature but according to dominancy of Dosha they can be classified according to Dosha. Pama Kushtha is one of the Shudra Roga which in Pitta-Kapha predominating.³ It is contagious and widely spreader disease throughout the world. Pama Kushtha characterized by many small Pitikas, Srava, Kandu, Toda and Daha that may be considered as scabies in contemporary science.⁴

Scabies is major public health problem with an estimated prevalence of 130 million cases worldwide. The prevalence varies from 0.35 to 46%.⁵ The highest rates are found in countries with hot, tropical climates, where infestation is endemic. Scabies is more prevalent in overcrowded communities with low socioeconomic conditions. The point prevalence in the general population of rural India is about 13%, In Maharashtra 81% of inmates of an orphanage in rural area,⁶ 72% of
cases are seen in the age group below 16 year. It may spread in a group of population within a short period of time due to close contact or skin to skin contact and contaminated clothes. Scabies, a common cause of itching is produced by infestation with the mite *Sarcoptes scabiei var hominis*. Poor hygienic condition and overcrowding, permitting close body contact favour the transmission of the disease. Mites of scabiei colonize the Horney layer of the human epidermis cause dermatitis. [8] *Acharaya Susruta* explained *Vachadi* Ointment in *Chikitsasthan* in the management of *Pama*. [9]

**Materials and Methods**

**Place:** *Kaumarbhritya* OPD of Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (Wardha).

**Case Report:**

A 10 years old male patient brought with complaints of itching in between the fingers of both the hands, wrists and forearms, Itching intensifies during night time, since 10-12 days.

**On Examination:**

1. Papulo-vesicular lesions with discrete presentation and distributed bilaterally over hands, wrists & forearms.

2. Multiple erythematous papules, Round shaped with a well-defined border measuring from 0.1 to 0.2 cm in diameter, with no scales.

3. Multiple spherical vesicles with a size range measuring from 0.2 to 0.3 cm in diameter with erosion that has already healed and crusted.

**History of present illness:**

According to his Father, patient was asymptomatic 1-2 week back. But since then patient had developed few rashes in between the fingers and started itching. This itchiness started from his fingers first which widespread to his wrist, forearm within last 6-7 days. Furthermore, he said that the itchiness worsens at night and his sleep was disturbed by intense pruritus. History of pyrexia was denied. They had consulted to nearest doctor and taken treatment for the same and got no satisfactory result then patient came to Mahatma Gandhi Ayurveda college hospital & Research Centre, Salod Wardha.

**Past History:**

Not significant

**Family History:**

Previously, his elder brother had experienced the same complaint. Also, he had a history of sharing the same room and bed with his brother.

**Table No.1 – Details of Personal History**

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Parameters assessed</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Appetite</td>
<td>Poor</td>
</tr>
<tr>
<td>2</td>
<td>Diet</td>
<td>Mixed (Veg &amp; Non-veg)</td>
</tr>
<tr>
<td>3</td>
<td>Bowel movements</td>
<td>Irregular 1-2 times a day</td>
</tr>
<tr>
<td>4</td>
<td>Urine</td>
<td>4-5 times a day</td>
</tr>
<tr>
<td>5</td>
<td>Sleep</td>
<td>Disturbed, wakes up crying at night</td>
</tr>
<tr>
<td>6</td>
<td>Likes</td>
<td>More of outside packed food, Biscuits &amp; Chocolates</td>
</tr>
<tr>
<td>7</td>
<td>Dislikes</td>
<td>Milk, dairy products</td>
</tr>
<tr>
<td>8</td>
<td>Nadi (Pulse): 78/ min.</td>
<td>Shabda (Speech): Clear</td>
</tr>
<tr>
<td>9</td>
<td>Mala (Bowel): mild constipation</td>
<td>Sparsha (Touch): Normal</td>
</tr>
<tr>
<td>10</td>
<td>Mutra (Bladder): Normal</td>
<td>Drika (Eyes): Normal</td>
</tr>
<tr>
<td>11</td>
<td>Jivha (Tongue): slightly coated</td>
<td>Akriti (Built): Madhyam</td>
</tr>
</tbody>
</table>
Diagnostic assessment:

The case was diagnosed on the basis of clinical presentation; it was diagnosed as *Pama Kashta* (Scabies).

Treatment Plan:-

Patient was treated on OPD basis.

### Table no. 2- Ingredients of Vachadi Ointment

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ingredients</th>
<th>Botanical Name</th>
<th>Part used</th>
<th>Quantity</th>
<th>1gm of Ointment contains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vacha</td>
<td>Acorus calamus Linn.</td>
<td>Rhizome</td>
<td>150gm</td>
<td>0.033gm</td>
</tr>
<tr>
<td>2</td>
<td>Daruharidra</td>
<td>Berberis aristata DC.</td>
<td>Rhizome</td>
<td>150 gm</td>
<td>0.033gm</td>
</tr>
<tr>
<td>3</td>
<td>Sarshap</td>
<td>Bressica nigra Koch.</td>
<td>Oil</td>
<td>4.5 Lt</td>
<td>0.825gm</td>
</tr>
<tr>
<td>4</td>
<td>Paraffin wax</td>
<td>-----</td>
<td>-----</td>
<td>200gm</td>
<td>0.117gm</td>
</tr>
</tbody>
</table>

### Table no. 3 - Properties of Vachadi Ointment

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Dravya</th>
<th>Karma/Action</th>
<th>Guna</th>
<th>Rasa</th>
<th>Virya</th>
<th>Vipak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vacha</td>
<td>Kapha - Vathar dipan, Truptighna, Rasayn, Asthapanopag</td>
<td>Laghu, Tiksha</td>
<td>Katu, Tikta</td>
<td>Ushna</td>
<td>Katu</td>
</tr>
<tr>
<td>2</td>
<td>Daruharidra</td>
<td>Shotahara, Varnashodhan, Varnaropan</td>
<td>Laghu, Ruksha</td>
<td>Kashay, Tikta</td>
<td>Ushna</td>
<td>Katu</td>
</tr>
<tr>
<td>3</td>
<td>Sarshap</td>
<td>Shotahara, Lekhan, Vednaathapan</td>
<td>Tiksha</td>
<td>Katu</td>
<td>Ushna</td>
<td>Katu</td>
</tr>
</tbody>
</table>

**Preparation of the Trial Drug:** The drugs will be prepared as per standard procedure mentioned in *Rasatarangini*; 

Vachadi Ointment will be prepared in Department of RSBK of Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha.

**Flowchart Of preparation Vachadi Ointment**

Course powder of *Vacha, Daruharidra & Sarshap* will be taken in Equal proportion

16 times water will be added to these drugs and heated on Mandagni till it is reduced to ¼ quantity to prepare a Quath.

Decoction will be filtered in steel container & Sarsap Tail in 1/8 quantity will be added and again heated on Mandagni till attains Sneha Siddhi Lakhanas.

Tail will be filtered and will be kept in an air tight container.

To this processed oil Paraffin-wax will be added in a proportion of 1:5 and stirred continuously till an ointment like consistency is obtained.
The ointment will be stored in an air tight container and dispatched in tubes measuring 20 gm each.

**Posology:-**

Treatment plan: *Vachadi* Ointment

Frequency: Thrice a day

Route: Local application

Site: Affected area

Dose: Quantity sufficient

Duration: 7 days

*Ahara and Vihara* (Diet and mode of life) advised during treatment:

**Table No-4: Pathya- Apathya**

<table>
<thead>
<tr>
<th>Pathya (Regimen to follow)</th>
<th>Apathya (Regimen to be restricted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahara</td>
<td>Ahara</td>
</tr>
<tr>
<td>Green gram, Rice, Wheat, Green vegetables, Fruits, Lukewarm water, Ginger water etc.</td>
<td>Ice cream, Cold drinks, Curd, Bread, Toast, Jam, Sauce, Non-Veg, Egg, Oily substances like chips etc, Fast food, Fermented foods, Sweet and sour taste foods, Milk, Yoghurt, Pickles etc.</td>
</tr>
<tr>
<td>Vihara</td>
<td>Vihara</td>
</tr>
<tr>
<td>Adequate sleep at night (8 hours), Maintain the personal hygiene, Clothes, bed linen, towels should be boiled and changed frequently, While bathing put few leaves of neem in the hot water.</td>
<td>Night awaking (Ratri Jagarana), Day sleeping (Diwaswapa)</td>
</tr>
</tbody>
</table>

**Observations & Results**

**Table No-5: Observations before and after complete course of treatment**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Symptoms</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kandu</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Toda</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Daha</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Srava</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Pidika</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure no-01**

(Before treatment)

**Figure no-02**

(After treatment)
Discussion

As per Ayurveda literatures, Pama Kushtha is Pitta-Kapha dominant Vyadhi and Kandu, Srava, Pidika etc. are the clinical feature. Vachadi Ointment having mainly Tikta, Kashaya Rasa, Ruksha, Laghu Guna, Sheeta Virya, Katu Vipaka hence they are Kapha-Pitta shamaka and acted as Vranaropaka, Shonitasthapaka, Kushthaghna. Externally application of Vachadi ointment for Shodhana (cleaning), Ropana (healing) of lesions, as well as Vranaropaka, Twachya property. Pathya-apathy and hygiene maintenance were also advised to patient. Child has followed the treatment protocol and Pathya-apathy properly and responded very well with treatment.

Conclusion

Hence it is concluded that external use of Vachadi ointment and proper hygiene maintenance are highly effective in the management of Scabies (Pama Kushtha).

Conflict of Interest – None

Funding Support – None

CTRI NO- CTRI/2019/08/020688

IEC No: DMIMS/DU/IEC/2017-18/7245

Acknowledgments- Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India

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Effectiveness of *Vachadi* Ointment in the management of *Pama Kushta* (Scabies): A Novel Research Protocol Using Science of Ayurveda

**Rakesh Khatana¹, Renu Rathi²**

¹PG Scholar; ²Prof. Department of Kaumarbhritya, Mahatma Gandhi Ayurved Collage Hospital & Research Centre, Salod, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India

**Abstract**

**Background:** Scabies is a common cause of itching is produced by infestation with the mite *Sarcoptes scabiei var hominis*. Poor hygienic condition and overcrowding, permitting close body contact favour the transmission of the disease. Scabies is more prevalent in overcrowded communities with low socioeconomic conditions. The point prevalence in the general population of rural India is about 13%, in Maharashtra 81% of inmates of an orphanage in rural area, 72% of cases are seen in the age group of School going children’s. *Pama Kushta* characterized by many small Pitikas, Srava, Kandu, Toda and Daha that may be considered as scabies in contemporary science. **Aim:** The present study is aimed at evaluating the effect of *Vachadi* Ointment in the management of *Pama Kushta*. **Material & Methods:** The present study is designed as a Non-randomized controlled clinical study, in which minimum of 30 patients will be enrolled. *Vachadi* Ointment will be administered external application 2-3 times in a day. Assessment will be recorded on 3rd, 5th, 7th and 14th days. **Results:** Changes will be observed in subjective parameters such as Kandu (Itching), Toda (Pain), Daha (Burning) and objective parameters such as Pitika (Eruption) and Srava (Discharge). **Conclusion:** Suitable conclusion will be drawn post completion of the trial.

**Keyword-** Poly herbal formulation, Pama, Scabies, Vachadi Ointment, Kandu, Pidika

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**Introduction**

Skin is largest and most important organ of the body as it protects the internal organs from the harmful environmental influences. Many agents can affect the skin they may be vary in nature - physical (trauma, heat), chemical (strong acids) and biological (variety of organisms). The skin has several inbuilt mechanisms for interacting with the environmental agents and most of the times the skin is able to protect itself from those agents. The protective ability however, may not always be able to deal with the environmental stimulus and this leads to the production of skin disease.[¹] Skin diseases in *Ayurveda* are described as *Kushta roga*. There are seven *Mahakushta* and eleven number of *Shudra kushta roga* are explained.[²] All *Kushta Roga* are *Tridoshaja* in nature but according to dominancy of *Dosha* they can be classified according to *Dosha*. *Pama Kushta* is one of the *Shudra Roga* which in *Pitta-Kapha* predominating [³]. It is contagious and widely spreaded disease throughout the world.

*Pama Kushta* characterized by many small *Pitikas, Srava, Kandu, Toda and Daha* that may be considered as scabies in contemporary science.[⁴] Scabies is major public health problem with an estimated prevalence of 130 million cases worldwide. The prevalence varies from 0.35 to 46%.[⁵] The highest rates are found in countries with hot, tropical climates, where infestation is endemic. Scabies is more prevalent in overcrowded communities with low socioeconomic conditions. The
point prevalence in the general population of rural India is about 13%.[6] In Maharashtra 81% of inmates of an orphanage in rural area,[6] 72% of cases are seen in the age group below 16 year. It may spread in a group of population within a short period of time due to close contact or skin to skin contact and contaminated clothes.[7] Scabies, a common cause of itching is produced by infestation with the mite Sarcoptes scabiei var hominis. Poor hygienic condition and overcrowding, permitting close body contact favor the transmission of the disease. Mites of scabiei colonize the Horney layer of the human epidermis cause dermatitis.[8] Acharaya Susruta explained Vachadi Ointment in Chikitsasthan in the management of Pama.[9] 

**Methodology**

**Trial design:** Non-Randomized Controlled Clinical Study

**Study setting:** Diagnosed Patients will be selected from Kaumarbhriya OPD & IPD of M.G.A.C.H. and R.C. Wardha.

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**Inclusion Criterion:**

- Diagnosed cases of Pama Kushta, irrespective of caste, religion, sex or socio-economic status.
- Children between the age group of 3 to 15 years.

**Exclusion Criterion:**

- Patients suffering from any other systemic disorders such as Skin Tuberculosis, Leprosy etc.
- Kushtha other than Pama/scabies.
- Pama associated with immunodeficiency disorder such as HIV.
- Scabies with crust or hyperkeratosis and Secondary infection, Urticarial etc.

**Criteria for discontinuing or modifying allocated interventions:** Subject will be withdrawal from the study if any untoward incidence, features of drug sensitivity or any other disease or problem arises, the subject will be offered free treatment till the problem subsides

**Follow up period after treatment:** 14th day after treatment.

**Primary Outcomes:** Changes in the symptoms of Gridhrasi such as Kandu (Itching), Toda (Pain) and Daha (Burning).

**Secondary Outcomes:** To observe the changes Pitika (Eruption) and Srava (Discharge)

**Statistical analysis:** The data will be analysed by using paired t-test & Wilcoxon rank sum test.

**Time duration till follow up:** The patient will be followed up during treatment 21 days.

Follow up period – 3rd, 7th and 14th

**Time schedule of enrolment, interventions:** Diagnosed patients of Pama Kushta (Scabies) will be enrolled in the present study after fulfilling the inclusion criteria.

**Interventions-** Table no-01 showing the Posology

<table>
<thead>
<tr>
<th>Treatment plan</th>
<th>Frequency</th>
<th>Route</th>
<th>Site</th>
<th>Dose</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vachadi Ointment</td>
<td>Twice a day</td>
<td>Local application</td>
<td>Affected area</td>
<td>Quantity sufficient</td>
<td>7 days</td>
</tr>
</tbody>
</table>

Sample size: 30
**Recruitment:** Patient will be recruited by single arm study

**Implementation:** Principal invigilator will allocate and enrol the patient.

**Data collection methods:** Non-Randomized

**Assessment criteria:**

**a) Subjective criteria:**

1) *Kandu* (Itching)

2) *Toda* (Pain)

3) *Daha* (Burning)

**b) Objective criteria:**

1. *Pitika* (Eruption)

2. *Srava* (Discharge)

**Table No-02**

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Grade Symptoms</th>
<th>0 Grade</th>
<th>1 Grade</th>
<th>2 Grade</th>
<th>3 Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kandu (Itching)</td>
<td>No Itching</td>
<td>Mild / Occasional Itching</td>
<td>Moderate / Frequent (every day) Itching</td>
<td>Severe (disturbed sleep &amp; other routine activities) Itching</td>
</tr>
<tr>
<td>2</td>
<td>Toda (Pain)</td>
<td>NO Pain</td>
<td>Occasionally Pain</td>
<td>Frequent (every day) Pain</td>
<td>Always (disturbed sleep &amp; other routine activities) Pain</td>
</tr>
<tr>
<td>3</td>
<td>Daha (Burning)</td>
<td>No Burning</td>
<td>Mild / Occasional Burning</td>
<td>Moderate / Frequent (every day) Burning</td>
<td>Severe (disturbed sleep &amp; other routine activities) Burning</td>
</tr>
</tbody>
</table>

**Table No-03**

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Symptoms</th>
<th>Grade-0</th>
<th>Grade-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pitika (Eruption)</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Srava (Discharge)</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

**Data management:** The data entry coding will be done by PI
Ethics and dissemination: Research ethics approval; approval from research ethics committee has taken no:- IEC No: DMIMS/DU/IEC/2017-18/7245

Consent or assent: The written assent will be taken from the patient before starting the study. During the study the confidentiality of each patient will be maintained.

Dissemination policy: The data will be disseminated in future clinical work and also paper publication.

Authorship eligibility guidelines and any intended use of professional writers

Informed consent materials: Model consent form and other related documentation will be given to participants and authorized informants with all the information.

Discussion

In the Pama there is predominance of Pitta and Kapha which leads to discolouration of skin like Shyava, Aruna and Shweta Varna Pidika associated with Pitta related symptoms like Daha, Toda and Kapha related like Kandu, Strava. The Vachadi ointment is mainly Tikta, Katu and Kashaya rasa predominant. All the drugs of Vachadi ointment are having Tikta and Katu Rasa which are having Aampachak, Raktaprasadaka, Kushtagha, Daha Prashamaka and Kaphahara properties so it is considered to be very much effective in the treatment of Pama Kushta. Laghu, Tikshna and Ruksha Guna subsides Kapha thereby increases Agni which helps in the removing Agnimidyaya and clears tiny Srotas by removing Aam. Laghu Guna of the compound helps in Lekhana and Poshana which has reduced the Pidika of Pama mainly. Tikta Kashya Rasa and Laghu Guna do the Lekhana Karma and Kapha alleviation too. Vachadi ointment by virtue of its natural properties alleviates Vata and reduces Kandu which is one of the main symptoms of Pama. Vachadi ointment has three drugs Vacha, Daruharidra and Sarshap which is indicated in Pama Kushta.

Expect Result

All the drugs have Antibacterial, Antiviral, Anti-inflammatory, Anti-allergic and Antimicrobial Activities. Vacha has wound healing and Anti-inflammatory properties. Daruharidra has properties of quickly healing wounds and has excellent drying quality which relieves moisture. Its paste applied externally can instantly relieve pain and inflammation. Daruharidra applied externally can relieve skin diseases and itching.

Conclusion

Pama in Modern parlance has similarity with the skin disease Scabies. Pama being Kshudra Kushta has Kapha-Pitta dominance with its involvement of Tridosha which can be evident by observing its signs and symptoms. The drug will be shown evidence to be a safe preparation in paediatric practice. The drug will be well accepted by children due to external application.

Conflict of interest – None

Funding Support – None

CTRI NO- CTRI/2019/08/020688

IEC No: DMIMS/DU/IEC/2017-18/7245

Acknowledgments- Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India

References


Palmar Dermatoglyphics and Idiopathic Epilepsy – A Systematic Review

Rashmi C Goshi
 Assistant Professor, Department of Anatomy, JSSMC, Mysuru

Abstract
Dermatoglyphics is the science which deals with the study of dermal ridge configuration on the digits, palms and soles. Etymologically this term is harmonious blend of two words Derma – skin; Glyphe – carve. It gives the impression that something has been carved out of the skin. The entire human body is clothed with the skin which happens to be the largest and most important organ of the body. However, the skin on the ventral sides of the hands and the plantar sides of feet is exclusively designed and is corrugated with the ridges and configurations which are functionally useful as they help in the grasping without which the objects would easily slip away from the hands. Dermatoglyphic traits are genetically determined. Dermatoglyphic abnormalities are due to genetic or other factors that express their effect before the end of 5th month of foetal development. The permanency of finger patterns, the extreme variability from one individual to the other and easy analysis are some of the reasons for its wide application in a variety of conditions. Abnormality in the genetic configurations of parents is inherited by children and is reflected in the dermatoglyphic pattern. Hence dermatoglyphic study proves to be a very useful, easily applicable, inexpensive, indispensable tool as an indicator in the diagnosis of hereditary diseases in patients. The etiology of the epilepsies allows a classification of syndrome features into two groups – idiopathic or cryptogenic epilepsy, which has isolated primary symptoms without apparent cause and is probably hereditary and finger print configurations are inherited with an embryonic origin common to nervous system. Their attractions indicate pleiotropic effects of the genotype responsible for enaphalographic irregularity and convulsive seizures.

Key words: Dermatoglyphics, Epilepsy, Finger prints.

Literature
Over the past 150 years, dermatoglyphics has been a useful tool in understanding basic questions in biology, medicine, genetics and evolution, in addition to being the best and most widely used method for personal identification.4 History of Dermatoglyphics. The scientific study of papillary ridges of hands and feet is credited as the beginning with the work of Joannes Evangelista Purkinje in 1823. William Herschel (1858) was the first to experiment with fingerprints in India. He noticed the use of thumb prints as a form of signature amongst illiterate Indians and clearly established the fact that fingerprints did not change their form over time. Sir Francis Galton (1892) published the book “Finger Prints” and in doing so, significantly advanced the science of finger print identification. He demonstrated the epidermal ridge configurations did not change throughout postnatal life. He divided fingertip patterns into three groups Arches, loops and whorls. Sir Edward Henry (1893) established the modern era of finger print identification. Morris Hawthorne Wilder (1902) pioneered comprehensive studies on methodology, inheritance and racial variation of palmar and plantar papillary ridge patterns, as well as fingerprints. Cummins and Midlo (1926) were the first to coin the term Dermatoglyphics. The main thrust of their research was on Down’s syndrome and the characteristic hand formations. Charles Midlo MD (1929) together with

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DOI Number: 10.37506/ijfmt.v15i1.13420
others published “Fingerprints of palms and soles”, a bible in the field of dermatoglyphics. Cummins (1935) proposed, that direction of epidermal edges was determined by growth forces and contour of volar skin at the time of ridge formation. Henry (1937) limited the designation of the term ‘whorl’ to those configurations having ridges that actually encircle a core. He named more complex patterns as ‘composite’. Penzose LS (1945) conducted dermatoglyphic investigations of his research into Down’s syndrome and other congenital medical disorders. Galton Center (1965) contributed to the development of dermatoglyphics and formulated the measurement to establish the position of displaced axial tri radius in terms of atd angle, as well as establishing the inheritance of its position in the palm. Sarah Holt (1968) published the book “The genetics of dermal edges” and summarized the statistical distributions of dermatoglyphic patterns in both normal and congenitally affected individuals. Hirsch and J.V. Schweichel (1973) emphasized that the neuroepithelium plays an important part in the development of dermatoglyphic patterns. Schaumann and Alter (1976) published a book “Dermatoglyphic in medical disorders” which summarizes the findings of dermatoglyphic patterns associated with congenital defects and significant markers of prenatal events. Babler (1987) reported that there is a relationship between the volar pad shape and the epidermal ridge configuration. He also suggested the association between the shape of the distal phalanx and the pattern type. Dr. Alexander Rodewald (2001) diagnosed many congenital abnormalities with 90% accuracy from the features of the hands. Dr. Stovens(2003), claims to diagnose schizophrenia and leukemia with 90% accuracy from the patterns on the hands alone. Cummins (1936) noted the significance of pad regression and initiation of epidermal ridge differentiation. In addition, recent embryologic studies have suggested that plantar, palmar and digital creases develop concurrently with volar pads[12,13] rather than as a consequence of early flexion movements. Development of epidermal ridges: Epidermal ridge morphogenesis has been reported by Kolliper. The critical period of primary ridge differentiation is between 11 and 17 weeks. This finding has been confirmed by Blechschmidt[25], Penrose and O’Hara[26], Okijima[27] and Babler. Epidermal ridges first appear as localized proliferations in the basal layer of the epidermis during the 10th week post fertilization. Primary ridges proliferate rapidly to keep pace with the increasing separation of adjacent ridges due to general growth of hand. This proliferation produces the branching and islands, the miniature. Hale[23] was able to show that the tendency of ridges to multiply was greatest during the period of maximum difference between the increase in surface area of hand and increase in ridge breadth. Primary ridges increase in width and penetrate deeper into the underlying dermis. Secondary ridges or furrow folds correspond to the furrow of surface ridge. Concomitant with secondary ridge formation is the termination of primary ridge formation. From 17 to 24 weeks, secondary ridges continue to proliferate and develop in a manner similar to that of primary ridges. At 24 weeks the epidermal ridge system has an adult morphology. Hirsch and Schweichel reported that ridge formation initiated along the laterodistal portion of the digit and proceeded in a proximomedial direction with the center of pad initially being free of ridges. Factors influencing ridge configuration: Current research suggests that genetic component of dermatoglyphic traits operates indirectly on ridge configuration through ontogenetic factors, eg: pad topography, growth rates and stress on the epidermis that influence ridge alignment. Epidermal ridge growth : From the initial appearance of primary ridges around 11 weeks, prenatal growth of primary ridges can be divided into 3 basic components – Width of the primary ridges Amount of penetration of the ridge into the dermis
3) Spacing or separation between adjacent primary ridges. Dermatoglyphic patterns form on finger pads prenatally and remain unchanged throughout the life. It can aid to the diagnosis of genetically and nongenetically determined diseases which cause distortion of patterns. Recently Penrose and O’Hara used electron microscopy to study the epidermal ridge development. Okijima and Miller reported the techniques to study surface ridges of the fetus. Personal identification:
Finger prints are constant and individualistic and can be the most reliable measure for personal identification. The secretions in the finger print contain residues and various chemicals and can be detected and used for forensic purpose and for criminal identification. It is important in medicolegal cases and disputed paternity positive identification using finger print can be established only if 16 – 20 points of similarity exist in the miniature. Concerning the distribution and degree of heritability of palmar main lines, it should be remembered that the differentiation of dermatoglyphics occurs during 3rd and 4th month of foetal life and that the lines remain constant after birth. Therefore except in size, the dermal configurations do not vary with age and are practically independent of environment effect.

Line A is considered to be an important indicator of the general direction of ridges coursing over a large area of palm (Wolt, Bsehane and Reinvein 1964). A-d ridge count has been used in pedigree studies. The mean count on left hand was significantly larger than on the right by approximately nine ridge (p<.01). This bilateral asymmetry of line A was evident in both sexes. (Cummins and Midlio 1961) Genes of additive effect ailing without dominance would be expected to give correlation coefficients of 0.5 for both parent child and sib-sib comparison (Fisher 1918). The theoretical correlation coefficient for child and midparent is 0.71 (Penrose 1949).

Calculation of the correlation coefficient between relatives for both the total finger ridge count (Holt 1961) and the a-b ridge count (Pons, 1964) have yielded values in close agreement with those expected on theoretical grounds for polymeric systems with genes of additive effect. The A-d ridge count appears to resemble the a-b and finger ridge count in that variation in this feature is largely under genetic control and subject to the influence of genes with additive effect. However conclusions are still contradictory. The results suggest a significant familial correlation (except spouse) indicating the involvement of familial component to the variation of dermatoglyphic traits. Segregation analysis reveals the transmission of genetic effects in the families which follows the Mendalian model. Major gene involvement with Mendalian expectation regarding finger dermatoglyphics is confirmed for all analyzed traits. However there is no evidence of significant support for major gene affect or environment effect on palmar a-b ridge count.

The history of dactylography as a fool proof tool for identity establishment has special significance in India – the first finger print bureau, established for medicolegal purpose in the world was at Calcutta. Today, digital dermatoglyphics form an indispensable and reliable tool for criminal investigation at internal level. Forensic scientists could extend their expertise and experience in dermatoglyphics to aid clinicians in medical diagnostic investigation.

Epilepsy is a chronic brain disturbance of varied and complex origin. Epileptic syndromes are classified mainly by brain location (generalized, partial or focal) and seizure type (clinic, tonic akinetic simple, absence or unconsciousness) which, together with electro-encephalographic studies, define the differential diagnostic (Delgado-Escueta et al 1982, Lancet 1990). The etiology of the epilepsies allows a classification of syndrome features into two groups – idiopathic or cryptogenic epilepsy, which has isolated primary symptoms without apparent cause and is probably hereditary. Second group is symptomatic epilepsy of genetic or acquired origin associated with other clinical features. Symptomatic epilepsy is due to cranial traumatism or as a result of infection, intoxication, brain tumours, hypoglycemia, drug abstinence etc. Jacksonian Seizures displayed only molar manifestation without impairment of consciousness and are typical among focal epilepsies. The genetic investigation carried out by Wenberg (1912) is of special historic interest because it was the first statistical segregational analysis of a human pathological condition. (McKusick 1992). Research on genetics of various epileptic syndromes has been reported and models designed for analysis of its familial segregation. (Anderson et al 1982) Isolated
idiopathic epilepsies with Mendelian inheritance do not encompass 5% of hereditary convulsive syndromes (Anderson and Hauser 1993) which are predominantly associated with chromosomal aberrations, metabolic disturbances and neurological deficiency. Other genetic molecular approaches involve neurotransmitters, inhibition of gamma amino butyric acid (GABA) synthesis in gerbils (Gerbillus) susceptible to epileptogenic factors (Fukuyama et al 1979) produced convulsive activity of similar origin to human epilepsy in the temporal lobe. (Osolsen et al 1984) Fere (1905) may have done the pioneer study on finger prints in epileptics. Many investigators reported several studies of finger prints in epileptics. Many investigators reported several studies of finger prints in individuals affected by non-specific types of epilepsy (Portius 1937; Brown and Paskind 1940; Katzenstein – Sutro 1945; Cherrill 1950; Alter 1966; Rosner et al 1967; Razavi 1975; Lopez and Lopez 1977; Karitono et al 1979; Schaumann and Mayerdorf 1979; Schaumann et al 1982; Cisarik et al 1985; Marinina and Drabktsina, 1988). Results revealed significant differences in loop frequencies in the left hand among male and female whole subjects. Considering separately each finger tip, the comparison between epileptic groups exhibited highly significant differences (p<0.01) in loop frequencies in right and in the left finger, the values being higher among generalized epileptics. The largest statistical differences were detected in finger III & I between epileptics and control. This suggests an epigenetic connection between embryonic regions I – III and normal physiology of CNS. Schaumann and Mayerdorf (1979) found an increase in radial loops in white adult patients with idiopathic epilepsy. In the non white group, the tented arch (At) pattern was completely absent in male generalized epilepsy and their control. Previous research among adult males with idiopathic epilepsy showed lower TRC values, although not significant at the 5% level of probability, comparatively to the controls (Schaumann and Mayerdorf 1979, Schaumann et al 1982). As in nearly all populations (Saldanha 1968), the TRC means were significantly higher (p<0.05) in male than in female groups for both epileptics and controls. The high evidence of arches on I & IV fingers, of raketoid loops on all fingers and especially IV & V, of the papillary ridges disposition as a dense and very dense network at thenar / I of the finalization of the line T’s direction of palm’s field 11 and 12 instead of 13 which contribute to enriching the clinical image of epilepsy’s dermatoglyphic diagnosis, at least for the patients living in Maldivian.

It has been opined that any epidermal ridge alteration in individuals prone to epilepsy may have a distinctive dermatoglyphic feature (Schaumann & Alter 1976). Idiopathic epilepsy of primary generalized epilepsy type is a tendency to have seizures when there is no structural abnormality in the brain. The primary cause could be genetic and a number of genes have been mapped Baulac et al (2001) and Brismar (2000). The dermatoglyphic traits, which presented a significant difference, were a-b ridge count, lateral deviation, palmar pattern and finger tip pattern. Mean values of a-b ridge count were more in epileptic patients, especially in left hand, than controls. The ratio of ulnar and radial deviations in control was 1:3 while in epileptics it was 1:5. Arch type of palmar patterns were showing a very significant difference between controls and cases. Frequency of loops was much more and vestiges were absolutely absent in cases. The frequency of Arch type of finger tip pattern was more in controls. A first and very important deviation at the level of affected people’s palm is a very strong reduction of models frequency in interdigital spaces IV & III. The strong decrease of distance a-b much below the average value was recorded in Romanian population. A higher frequency of cases was observed in which the papillary ridges from Thenar / I are arranged as a dense or very dense network.

Abnormalities of early development including genetic disorders. On literature review, it was noticed that hardly few studies have reported the association between dermatoglyphics and idiopathic epilepsy. Authors have found two significant variables in males diagnosed with idiopathic epilepsy. (i) decreased ‘a-b’ ridge count on both palms (p<0.1) and (ii) more frequent existence of transverse sulcus. The mean age of onset of epilepsy was 15.8 years in males and 13.9 years in females. 18.4% of the male patients and 10.2% of female patients were products of consanguineous marriage; 12% of male and 22% of female patients had family history of epilepsy. Various studies have shown different variations in the qualitative and quantitative parameters of palmar dermatoglyphics in Epilepsy patients.
Priya Ranganatha found no significant difference in the a-b ridge count between patients and controls in males and females. Difference in ‘atd’ angle between patients and controls in males and females was not found to be significant.\textsuperscript{19} A Study on finger tip patterns on comparison of epileptics with controls, in males, with hands combined, loops and arches were increased and whorls were decreased (\(p<0.05\)). In females, with hands combined, arches and whorls were increased and loops were decreased (\(p<0.03\)). Significant differences have not been observed for the patterns in hypothenar area / interdigital area and flexion creases.\textsuperscript{21} The authors distinguished correlation between dermatoglyphic features and form of epilepsy, type of course and pathogenic forms.\textsuperscript{22} Dermatoglyphics characteristic in epileptic patients varied in groups with different clinical characteristics (age at the onset of disease and the duration of the latter, the daily development of paroxysms at the same time, a tendency toward a stable course, the presence of psychic states and the resistance to therapy). Statistical task solving rules for diagnosing epilepsy in children were elaborated. The correct computer aided diagnosis on the basis of dermatoglyphic examination was made in 70\% of epileptic patients.\textsuperscript{23} A dermatoglyphic study of adult Caucasian males with a confirmed diagnosis of epilepsy was carried out. A multivariant analysis was employed on an enlarged patient sample. Variables found to be significant were decreased a-b ridge count on both left and right palms (\(p<0.001\)). Tests of eigenvalues showed only one value to be significant and accounting for 71.8\% of the intergroup variation.\textsuperscript{24} The data are important for medical genetic consultation and provide information for the theory of genetic issues in epilepsy.\textsuperscript{25} Significant findings in qualitative analysis of male epileptic patients showed increase in frequency of radial loops on finger tips of right and left index fingers and decrease in frequency of whorls on right thumb, index, ring and left ring fingers. In female epileptics there was increase of inter-digital fourth pattern VIII in both hands. Significant findings in quantitative analysis of epileptic patients included increase of total finger ridge count in male and female epileptics.\textsuperscript{28} Authors reported a k289m mutation in GABAA receptor \(\gamma_2\)-subunit gene (GARG2) that segregates in a family with a phenotype closely related to GEFS+, an autosomal dominant disorder associating febrile seizures and generalized epilepsy previously linked to mutations in sodium channel genes, thus providing the first genetic evidence that a GABAA receptor is directly involved in human idiopathic epilepsy.\textsuperscript{29} The comparison of palmar pattern types revealed significant difference between symptomatic and controls for right second inter digital area and left hypothenar area. Digital pattern intensity index was higher in epileptics than controls.\textsuperscript{31} The significantly increased prevalence of dermatoglyphic abnormalities in epileptics and in their parents suggests a polygenic mode of inheritance. Thus the view that convulsions are inherited by a single dominant gene with incomplete penetrance needs to be reexamined.\textsuperscript{32}

**Conclusion**

One of the most important parameter of dermatoglyphics is the inheritance. Understanding of development of dermatoglyphic traits requires to view it as a living history of prenatal development. Hence it is important in the medicolegal cases of disputed paternity. It has very crucial role in the diagnosis of Monozygotic and dizygotic twins. Degree of affection of investigated epileptics is correlated with the presence in the palmar dermatoglyphics of significant variations.

**Ethical Clearance:** Taken from ethical committee.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Comparison of the Serum Concentrations of Micronutrients Zinc and Iron in Epileptic Children before and after Administration of Valproic ACID

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Abstract

Introduction: The mortality rate of epileptic patients has increased despite advances in the treatment of epilepsy. Several studies have indicated that changes of micronutrient homeostasis led to seizures. The anticonvulsant valproic acid affects the intracellular concentration of micronutrients.

Objective: To compare the serum concentrations of micronutrients zinc and iron in paediatric patients with epilepsy before and after administration of valproic acid.

Method: A prospective cohort design study was conducted in children with epilepsy from July to December 2019 in the outpatient department of paediatric neurology, Dr. Soetomo Regional Public Hospital, Surabaya, Indonesia. Serum concentrations of micronutrients zinc and iron were measured using the colorimetric method. Statistical analysis used paired t-test with p<0.05 being considered significant.

Results: The study sample comprised 20 children with epilepsy. There were 13 (65%) males. Eight (40%) children were in the 1-5 year age group. Seizure type was generalised in 18 (90%) patients. The mean serum iron concentrations before and after valproic acid treatment were 23.9±16.8 µmol/L and 25.8±16.4 µmol/L respectively (p=0.700). The mean serum zinc concentrations before and after valproic acid treatment were 13±16.8 µmol/L and 26.8±15.4 µmol/L respectively (p=0.004).

Conclusions: There was a statistically significant increase in the mean serum zinc concentration after valproic acid treatment in children with epilepsy. There was however no statistically significant increase in the mean serum iron concentration after valproic acid treatment in children with epilepsy.

Key words: epilepsy, micronutrients iron and zinc, valproic acid, children.

Introduction

Globally, 50 million people are affected by epilepsy with more than half of the cases occurring in children¹.

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In Indonesia, there are 700.000-1.400.000 cases of epilepsy, with 70.000 new cases every year and about 40-50% of these are children². Valproic acid affects intracellular zinc concentration, the zinc transporter (ZnT1) playing a role in intracellular zinc homeostasis. Serum zinc concentration may decrease as a result of valproic acid, being affected by the dietary intake of zinc, stress, serum albumin concentration and the metabolic state of the patient³. An alteration of iron metabolism, consisting of an increase of non-transferrin bound iron (NTBI), occurred in 24 epileptic patients receiving valproic acid monotherapy⁴. This study was conducted
to observe the effect of anti-epileptic drug valproic acid on the serum concentrations of zinc and iron.

**Objectives**

To compare the serum concentrations of micronutrients zinc and iron in paediatric patients with epilepsy pre- and post-administration of valproic acid.

**Method**

A prospective cohort observational analytical study was conducted from July to December 2019 in children aged 18 years or less with newly diagnosed epilepsy in the Outpatient Department of Paediatric Neurology, Dr. Soetomo Regional Public Hospital, Surabaya, Indonesia, using valproic acid monotherapy. The characteristics of the study population (age, sex, seizure type, laboratory examination and electroencephalogram (EEG) were recorded. Serum concentrations of micronutrients iron and zinc were measured using the colorimetric method. Statistical analysis used paired t-test and p<0.05 was considered significant.

A written statement of agreement was obtained from the parents or guardians for the child’s participation in the study. Ethics Approval was obtained from Health Research Ethical Committee of Regional Public Hospital of Dr. Soetomo Surabaya before commencing the study.

**Results**

The study sample comprised 20 children. The basic characteristics of the study sample are shown in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex - n (%)</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Male</td>
<td>07 (35)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Age in years – mean ± SD</td>
<td>4.2 ± 4.23</td>
</tr>
<tr>
<td>&lt; 1 year - n (%)</td>
<td>05 (25)</td>
</tr>
<tr>
<td>1-5 years - n (%)</td>
<td>08 (40)</td>
</tr>
<tr>
<td>&gt;5 years - n (%)</td>
<td>07 (35)</td>
</tr>
<tr>
<td>Nutritional status - n (%)</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Normal</td>
<td>07 (35)</td>
</tr>
<tr>
<td>Malnutrition</td>
<td></td>
</tr>
<tr>
<td>Type of seizure - n (%)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>General</td>
<td>02 (10)</td>
</tr>
<tr>
<td>Partial</td>
<td></td>
</tr>
<tr>
<td>Family history of epilepsy - n (%)</td>
<td>05 (25)</td>
</tr>
<tr>
<td>Yes</td>
<td>15 (75)</td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl), mean ±SD</td>
<td>11.4 ± 1.29</td>
</tr>
<tr>
<td>Normal</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Electroencephalogram (EEG) - n (%)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Normal</td>
<td>09 (45)</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
</tr>
</tbody>
</table>
Of the 9 cases with abnormal electroencephalograms (EEGs), 3 had benign rolandic epilepsy.

Serum iron and zinc levels before and after valproic acid therapy are shown in Table 2.

### Table 2: Serum iron and zinc levels before and after valproic acid therapy

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Serum iron levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥24 µmol/L)</td>
<td>10 (50)</td>
<td>23.9 ±16.8</td>
</tr>
<tr>
<td>Low (≤8 µmol/L)</td>
<td>06 (30)</td>
<td></td>
</tr>
<tr>
<td>Normal (8-24 µmol/L)</td>
<td>04 (20)</td>
<td></td>
</tr>
<tr>
<td>Serum zinc levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥20 µmol/L)</td>
<td>04 (20)</td>
<td>13 ±16.8</td>
</tr>
<tr>
<td>Low (≤11 µmol/L)</td>
<td>10 (50)</td>
<td></td>
</tr>
<tr>
<td>Normal (11-20 µmol/L)</td>
<td>06 (30)</td>
<td></td>
</tr>
</tbody>
</table>

The mean serum iron concentration before treatment was 23.9 ± 16.8 µmol/L and the mean serum zinc concentration before treatment was 13 ±16.8 µmol/L. The mean serum iron concentration after valproic acid treatment was 25.8 ±16.4 µmol/L and the mean serum zinc concentration after valproic acid treatment was 26.8 ±15.4 µmol/L. The results of the analysis using paired t-test showed a significant difference between the mean serum concentrations of zinc before and after valproic acid treatment in patients with epilepsy (p = 0.004). However, there was no significant difference found between the mean serum concentrations of iron before and after valproic acid treatment in patients with epilepsy (p = 0.700).

The correlation between anaemia and serum iron and zinc levels after valproic acid therapy is shown in Table 3.

### Table 3: Correlation between anemia and iron and zinc levels after being treated with valproic acid

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Serum iron levels after valproate acid therapy</th>
<th>p*</th>
<th>Serum zinc levels after valproate acid therapy</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin Anaemia</td>
<td>1</td>
<td>7</td>
<td>10</td>
<td>0.558</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 was considered statistically significant; Chi Square test was used
Correlation of serum iron and zinc levels before and after valproate acid therapy is shown in Table 4.

**Table 4: Correlation of serum iron and zinc levels before and after valproate acid therapy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before and after valproate acid therapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron levels (mean ± SD)</td>
<td>1.90 ± 21.8</td>
<td>0.700</td>
</tr>
<tr>
<td>Serum iron levels (mean ± SD)</td>
<td>13.79 ± 18.6</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

*p <0.05 was considered statistically significant*

**Discussion**

In this study of epileptic children 65% were males. This was similar to the study by Serdaroglu et al (2004) where there was male predominance. In our study 40% were in the 1-5 year age group. Topbas et al (2012) also found most epileptics to be 1-5 years old. In our study 50% of patients were anaemic. Zareifar et al (2012), reported that haemoglobin concentration was significantly lower in children with epilepsy. In our study only 25% of patients had a family history of epilepsy. This is similar to the study by Omar et al (2016) where there was a family history of epilepsy in 25% cases. In our study 90% had generalised seizures. In the study by Andrianti et al (2016) generalised seizures were the predominant type. EEG examination is vital to confirm the clinical diagnosis of epilepsy, classification of epilepsy type, epileptogenic focus, treatment evaluation, and prognosis determination. However, the first EEG was normal in 55% of our cases. However, it should be remembered that the EEG was done when the patient was not in seizure (inter-ictal). In our study 35% of children with epilepsy were malnourished. In a study by Hardaningsih et al (2016) malnutrition status based on WLZ score <-2SD proved to be one of status epilepticus risk factors in children with seizure.

The zinc status in man is measured by the zinc concentration in plasma and serum. However, this is not a good indicator since less than 1% of the zinc total in the body is circulating in the plasma and also because the plasma zinc concentration is affected by hypoalbuminemia, haemoconcentration and acute phase response. Children in developing countries received inadequate zinc intake. A study by Soltani et al (2016), explained that zinc concentration decreased in children with epilepsy before starting administration of anti-epileptic drugs. In our study, children with epilepsy, prior to receiving valproate acid treatment, had low serum zinc concentrations in 10 (50%), normal serum zinc concentrations in 6 (30%) and high serum zinc concentrations in 4 (20%). The mean zinc concentration was 13±16.8 μmol/L.

A study conducted in man about valproate acid mechanism in zinc metabolism remains controversial. This study obtained a mean serum zinc concentration of 26.8 ±15.4 μmol/L after the administration of valproate acid. This indicated an increase of zinc concentration compared to that before the administration of valproate acid. High concentrations were obtained in 14 (70%) patients and normal concentrations in 6 (30%) patients. Two patients had the same high zinc serum concentrations before and after the administration of valproate acid. Analysis using paired t-test showed a significant difference of mean serum zinc concentration before and after administration of valproate acid. Analysis using paired t-test showed a significant difference of mean serum zinc concentration before and after administration of valproate acid. This study also found normal zinc serum concentration, as a result of 1% zinc serum concentration from all zinc of the body and did not describe the state...
of total zinc in the body. According to Verrotti et al. (2002), the effect of long term administration of valproic acid treatment in 36 patients with epilepsy before and after the 1 year-treatment showed that zinc concentration was normal.

In our study, children with epilepsy, before the administration of valproic acid had low serum iron concentrations in 6 (30%) and normal serum iron concentrations in 14 (70%) patients. The mean serum iron concentration in children with epilepsy before administration of valproic acid was 23.9 ± 16.8 µmol/L and mean serum iron concentration after administration of valproic acid was 25.8±16.4 µmol/L. This indicated an increase of iron serum concentration before and after the administration of valproic acid treatment. However, in this study, after the administration of valproic acid, serum iron concentration was normal in 12 patients, low in one patient and high in 7 patients. From the paired t-test, it was shown that there was no significant difference in mean serum iron concentrations before and after valproic acid treatment in patients with epilepsy (p = 0.700).

The serum iron level in each individual may vary 10-40% because of the change in iron absorption, bone marrow uptake on iron, or iron deposit expenditure. Besides, iron serum concentration is determined by iron absorption in the intestines, iron deposit, the speed of haemoglobin breakage and new haemoglobin formation.

The increase in serum iron concentration after valproic acid treatment resulted from change in iron homeostasis; consequently, the free iron in plasma or non-transferrin-bound (NTBI) iron began to accumulate in the blood and this resulted in increased serum iron concentration. The NTBI iron was finally inserted in the tissue by an unknown mechanism, which caused the impairment of cells and tissues. In most patients, excessive iron was a result of NTBI uptake from the circulation. Similar results were found in the study by Ounjaijean et al. (2011), stating that 24 patients with epilepsy receiving VPA monotherapy caused an iron metabolism change, therefore an increase was found in NTBI. In our study, mean serum iron concentration was normal after valproic acid treatment, similar to the study by Soltani et al. (2016), where serum iron concentration did not change.

Conclusions

Serum concentration of iron and zinc on pediatric patients with epilepsy increased after valproate acid treatment.

Conflict of Interest: The authors declare that there is no conflict of interest regarding this research.

Source of Funding: The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Clearance: This study was approved by the Ethical Committee of Dr. Soetomo General Hospital, Surabaya No. 1547/KEPK/X/2019

References


Palashkshar ointment in the management of Abhyantar Gudarsh – An Ayurvedic Management Protocol

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Abstract

Background: Hemorrhoids or piles are a common ailment among adults. More than half of men and women aged 50 years and older will develop hemorrhoid symptoms during their lifetime. This disease Gudarsha comes under the heading of Mahagada, as it is Dirghakalanubandhi, Dushchikitsya in nature, Tridoshaj and involves the Marma and it is well known for the chronicity and difficult to treat. Aim & Objectives: To evaluate the effect of local application of Palash Kshar Ointment in Management of Abhyantar Gudrsha.

Methodology: The study is prospective randomized controlled clinical trial. Total 60 patients will be selected for this study, which will be further divided randomly into two groups. Each group consists of about 30 patients. In which group A, will be trial groups and group B will be control groups. Every patient will be observed at regular follow up. On 7th, 14th, 21th, 28th Day. Results– Results will be drawn from the observations of objective parameters. Conclusion – Conclusion of the study will be drawn on the basis of statistical data calculated from the collected data.

Keywords:- Internal Haemorrhoid, Abhyantar Gudarsh, Palashkshar Ointment, Palash Kshar.

Introduction & Rationale

According to Acharya Sushruta, due to Hetu (like Viruddha – Aahar, Adhyashana, Stree- Prasanga, Utkatasana, Prustha – yaan, Veg – Vidharan etc.) Dosha Prakop occurs. The main Hetu is Mandagni, which is mentioned as ‘Visheshto Mandagne’. These prakupeet Doshas alone or all together with or without Rakta, enters in the pradhan Dhamani (main channel), go downward and reach at Guda. By vitating the Gudavalies, Produces the Mansa-Prarohas are known as Arsha1. The management of abhyantar Gudarsha i.e. Internal haemorrhoid is mainly divided in four types:- Bheshaj Chikitsa (Medicinal Treatment), Ksharchikitsa, Agnikarma (Cauterisation), Shastrakarma (Surgery).

In application of Kshar, intervention of doctor is necessary and compulsory. To make the process convenient for patient to apply the Kshar at internal haemorrhoid and to avoid doctor’s intervention for application of Kshar, Concept of application of Kshar by patient himself is presumed and for this purpose the Ointment of Kshar is preferred.

Therapeutic treatment of haemorrhoids ranges from dietary and lifestyle modification to radical surgery, depending on degree and severity of symptoms.2,3 The primary objective of most topical treatment aims to control the symptoms rather than to cure the disease. The number of topical preparations contains various ingredients such as local anaesthesia, corticosteroids, antibiotics and anti-inflammatory drugs.4 No radical topical is available. Hence radical non surgical treatment
form of topical ointment is need of time.

Although effective but traditional Pratisarniya Ksharkarma is more complex and tedious process. So modification in it is necessary. Kshar ointment is modification of Kshar Pratisaran. Previous study with Yava Kshar ointment was done. Siktha tail is previously used as a base for Kshar ointment preparation is proven safe and effective as a base. This showed encouraging results. But Yava Kshar is difficult to prepare and also expensive. Palash kshar is used as Pratisarniya Kshar at many places and is very effective. Hence Palash Kshar is used in current study in place of Yava Kshar. Palash Kshar ointment which is an ointment of Palash Kshar with base Siktha taila is modified technique for Ksharkarma which will be used in current study.

**Purpose of the study:-**

1. Number of patients for per rectal bleeding secondary to internal haemorrhoids are more. Topical ointment as a radical treatment is not available hence its complete study is necessary and feasible.

2. Palash kshar is used from years as a Pratisarniya kshar for management of internal haemorrhoids. Hence it is ethical to use Palash kshar Ointment in the management of internal haemorrhoids.

3. In application of kshar, intervention of doctor is necessary and compulsory. To make the process convenient for patient to apply the Kshar at internal haemorrhoid and to avoid doctor’s intervention for application of kshara, concept of application of Kshar by patient himself is presumed and for this purpose the Ointment of Kshar is preferred.

4. Haemorrhoid is disease associated with per rectal bleeding which is one of major reason for anaemia and ultimately blood transfusion. Many patients refuse for surgery due to their personal reasons and wants conservative management. So interest is developed in radical management of internal haemorrhoids using local application of Kshar ointment

**Aim –**

To study effect of Palash Kshar Ointment in management of Abhyantar Arsha.

**Research Question**

Whether Palashkshar ointment is effective in Abhyantar Arsha Chikitsa to reduce per rectal bleeding and size of internal haemorrhoids?

**Hypothesis**

Hypothesis of this PhD thesis will be -

*Palash Kshar* ointment applied locally at internal haemorrhoids once a day for 14 days is effective in reducing per rectal bleeding and degree of internal haemorrhoids.

**Hypothesis**

**H0** = *Palash Kshar* ointment applied locally at internal haemorrhoids once a day for 15 days is not effective in reducing per rectal bleeding and degree of internal haemorrhoids.

**H1** = *Palash Kshar* ointment applied locally at internal haemorrhoids once a day for 15 days is effective in reducing per rectal bleeding and degree of internal haemorrhoids.

**Study Design**

**Literary Review**

Review of Ayurvedic Literature
Review of Modern Literature
Review of Previous Work Done
Identification of Palash (Botanists)

**Preparation of Drug**

Preparation of Palash Kshar
Preparation of Palash Kshar ointment
Phyto Chemical Analysis of Palash Kshar

**Clinical Study**

Observation

**Assessment**

Statistical Analysis
Discussion
Conclusion

**Figure 1: Flow diagram of the study procedure**
Clinical Study

Figure 2 Flow diagram of Clinical Study

Methodology

1] Type of Study Design :-

Randomized open labelled controlled clinical study

2] Location of Study:

Clinical Study:- Patients will be randomly selected from the OPD of the Dept. of Shalya Tantra of M.G.A.C.H & R.C. Salod (Hirapur), Wardha. A written informed consent will be obtained by counseling the patients of Arsha (Internal Haemorrhoids) for participation in the study. The selection of method of patient will be Lottery method of Simple Random Sampling.

Drugs:- Drug Name:- Palash (Butea Monosperma)

Part used - The leaves, Flowers, Seeds, gum and stem. The parts will be collected in their respective Dravya-Grahan Kaal. The trial drugs will be collected from the local area and certified by Foundation for Revitalisation of Local Health Traditions, Banglore (FRLHT) and the Standardisation of Palashakshara will be done in Pharmacy of the Institute of M.G.A.C.H & R.C. Salod (Hirapur), Wardha.
3] **Duration of Study:** In the schedule of Ph.D course. (3 year)

4] **Plan of study:**

A) **Kshar ointment**

1) Preparation of ointment base *Siktha Taila* 7

2) Preparation of Ointment.

   a. Preparation of *Palash Kshar* Ointment base *Siktha taila* i.e. *Palash Kshar* 1 part and *Siktha taila* 5 part.

**Methodology**

The study is prospective randomized controlled clinical trial. Total 60 patients will be selected for this study, which will be further divided randomly into two groups. Each group consists of about 30 patients. In which group A, will be trial groups and group B will be control groups.

**Selection criteria for patients:**

- The patients having clinical symptoms of internal hemorrhoids will be included in study irrespective of sex, religion, diet habit.
- Patients having age between 21 years to 70 years.
- The patients of IInd and IIIrd degree internal haemorrhoids will be included in this study.

**Rejection Criteria for patients:**

- The patients of piles having previous history of haemorrhoidectomy.
- Pregnant woman will not include in this study.
- Patients taking other treatment from any pathya for the same problem during clinical trial.
- Patients who are suspected of serious systemic disease i.e. DM, TB, malignancy, Syphilis or having portal obstruction
- Patients Suspected of carcinoma of rectum, proctalgiafugax, chron’s disease, fistula in ano & where it is contraindicated
- Patients with severe anaemia i.e haemoglobin less than 5%

**Withdrawal Criteria for patients:**

- The patients not following protocol of study.
- Patients requiring emergency treatment or surgical treatment or other treatment
- Patients not ready to continue treatment.

**Treatment allowed for patients:**

- Patients requiring treatment for hypertension or other life saving activity
- Haematronics, Blood transfusion.
- Any short term treatment not more than 5 days for sudden onset disease like fever, which will not disturb protocol of study.

**Methodology**

**Group A : Study Group**

Local Application of *Palash Kshar* Ointment with Pathya

**Group B : Control Group**

*Palash Kshar Pratisaran* with Pathya

**Pathya for both Groups**

- Hot sitz bath twice a day
- Advice the patient to keep the local site hygienically clean.
- Laxative – Avipattikar Churna 2-6 gm at bedtime with lukewarm water (Dose will be adjusted as per requirement of patient. when constipation waill not be relieved dose may be increase).
- Dietary control will be advised.

**Treatment of subject**

**Period**

**Group A : Trial Group** – Once a daily for 14 days.

**Group B : Control Group** – Once a week for 4 weeks

**Dosage for both groups** – as required depending upon surface.

**Dosage schedule** – After defecation in morning once a day.

**Route of administration** – Topical (Locally on internal haemorrhoids)
Procedure for local application of Palash Kshar ointment:

Patient will be taken for local application after Hot sitz bath patient will be given lithotomy position i.e the patients is positioned supine comfortably with the knees flexed and abducted and buttocks projection well over the edge of table. The legs should be flexed at the hips and knees. Then local application at haemorrhoids will be applied. After that, a sterile pad kept at the anal verge to avoid soilage of clothes.

Procedure for local application of Palash Pratisarniya Kshar:

Patient will be taken for local application after Hot sitz bath patient will be given lithotomy position. The Kshar karma will be performed with the help of slit-type proctoscope under local anesthesia. Kshar will be applied on internal hemorrhoids one after the other. After application on each hemorroid, the applied Kshar will be cleansed with lemon juice after changes in colour approx one to three minute. Yashtimadhu ghruta is applied over haemorrhoids. After that, a sterile pad kept at the anal verge to avoid soillage of clothes.

Assessment –

Graduation of Internal Haemorrhoids (As per Goligher): -

I - Haemorrhoids projecting slightly in lumen of anal canal, when veins are congested at defecation.

II - Haemorrhoids prolapse out of the anus on straining, but return spontaneously to the anal canal when motion has been passed and the defection has ceased.

III - Haemorrhoids prolapse but don’t reduce spontaneously and remain prolapsed afterwards and have to be replaced digitally.

IV - Completely irreducible haemorrhoids, usually are long standing and acquire a component of skin.

P/R Bleeding grade:

I Grade – 0 to 5 drops

II Grade – 6 to 15 drops

III Grade – 16 and above drops

Discharge –

Severe - Changing sanitary pads or cotton pads minimum 2 times a day

Moderate - Changing of pads once a day only

Mild - No requirement of pads

Nil - Area is completely dry

Complication Assessment

To assess anal pain -

Severe - To relieve from pain, analgesic injections are required/Pain or discomfort dose not reduce after oral analgesics.

Moderate - To relieve from pain, oral analgesics are required.

Mild - Feeling discomfort within tolerable limit, no requirement of analgesic either orally or in other route.

Nil - No discomfort in any manner in the site, no analgesics.

Tenderness standard assessment:

Severe - Patient feeling pain by touching perianal area. Not possible to perform P/R examination.

Moderate - Little finger P/R can be done, patient feeling very much tolerable pain

Mild - Index finger P/R done with very much tolerable pain

Nil - Index finger insertion to anal canal without any pain or discomfort

Every patient will be observed at regular follow up. On 7th, 14th, 21st, 28th. For visual recording the regular photographs of selected patients from each group will be taken for observing the local changes at internal haemorrhoid.

Routine investigations of all patients and some specific investigations will be carried out as and when required.
A. Lab Investigations -

Laboratory Investigations will be carried out before including the patient’s under the study to rule out any other pathological conditions.

a. Routine Hematological Investigation –
   a. Complete Blood Count
   b. Random Blood Sugar

b. If Required:
   a. Urine Examination - Routine & Microscopic
   b. Stool Examination - Routine & Microscopic
   c. Biochemical Examination –
   d. Histopathological Investigation

Criteria for assessing the Result:

Completely Cured: Complete relief in all parameters

Improved: Improvement in 1 or 2 parameters

Uncured: When there was no improvement in any parameter.

Complication: When there was any complication due to hypersensitivity reaction to ointment (i.e. Itching, Severe pain, infection)

Observation

Maintained records & analyzed data collected after the study will be the source of observation & results drawn consequently will be discussed in the dissertation.

Statistical Analysis

Statistical Analysis will be done on observed results irrespective of sex, religion, socio-economic status, occupation, etc.

The confidence limit will be fixed at 95% and the level of significance will be at 5%. Paired and unpaired ‘t’ test will be applied for objective parameter and chi square test will be applied for subjective parameter. If required Mann Whitney U test may be applied for subjective parameter.

Discussion

After obtaining the analyzed data Discussion will be done according to Ayurvedic and Modern text will be done.

Figure no. 1 Gnatt Chart (Quarterly based)

<table>
<thead>
<tr>
<th>Item</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>Q6</th>
<th>Q7</th>
<th>Q8</th>
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<td>Enrollment of Volunteer</td>
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<tr>
<td>Writing thesis up to methods</td>
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<td>Data analysis</td>
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<tr>
<td>Writing thesis up to results and Conclusions</td>
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</tbody>
</table>
Conclusion

On the basis of available literature, observations & discussion conclusion will be precisely drawn and whole dissertation work will be summarized in the final dissertation.

References


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Response Evaluation on Single Common and Uncommon EGFR Mutation on First-Generation EGFR-TKI Therapy in NSCLC Patients

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Abstract

Objective: To compare the response of first-generation EGFR-TKI (epidermal growth factor receptor-tyrosine kinase inhibitors) in non-small cell lung cancer (NSCLC) patients with single common and uncommon EGFR mutation.

Methods: Patients were divided into two groups, the uncommon (exon 21 L861Q, exon 18 G719X, exon 18 delE709) and common EGFR mutation group (exon 19 deletion, exon 21 L858R). Health-related quality of life (HRQOL) using EuroQol EQ-5D® questionnaire, body weight, performance status (PS), Response Evaluation Criteria in Solid Tumors (RECIST) on chest CT, progression-free survival (PFS) and overall survival (OS) was recorded during TKI therapy.

Results: The value of HRQOL was stable and PS was constant in both groups, body weight was constant in uncommon group (42.1%) and increased in common group (44.1%; p=0.165). The uncommon group showed mostly progressive disease in RECIST (47.4%) while the common group showed mostly partial response (42.2%; p=0.007). PFS in the uncommon group was 4 (2.0-6.0) months and 7.0 (2.0-21.0) months in the common group (p=0.001). OS in the uncommon and common group were 4.00±1.71 months and 10.00±6.94 months (p<0.001), respectively.

Conclusion: NSCLC patients with common EGFR mutations showed a better response and survival rate compared to uncommon EGFR mutations on first-generation TKI therapy.

Keywords: NSCLC, EGFR mutation, tyrosine kinase inhibitor, uncommon, common.

Introduction

Lung cancer is a leading cause of cancer-related death worldwide. In 2018, more than 1.6 million individuals were diagnosed with lung cancer and five-year survival is roughly 17% for all stage1-2. There are substantial evolutions in the management of non-small cell lung cancer (NSCLC) in the most recent decade. Analysis of immunology and genomic tumor biomarker have now become a regular examination in NSCLC, notably adenocarcinoma. One of the gene alteration examinations that has been generally applied is the
epidermal growth factor receptor (EGFR) mutation. About 90% of all EGFR mutations are deletion of exon 19 deletion and point mutation L858R in exon 21. Meanwhile, less-common EGFR mutations, likewise called “uncommon”, “rare”, “nonclassical”, or “minor” are about 10% of all EGFR mutations. They might comprise of insertion at exon 20, point mutation at exon 18 or compound mutations².

EGFR tyrosine kinase inhibitors (TKI) is one of the significant discoveries in the treatment of lung cancer. Longer progression-free survival (PFS), a better quality of life and lighter drug side effects were seen in patients given first-generation EGFR-TKI compared to patients receiving standard chemotherapy. Most patients with EGFR mutations respond well to EGFR-TKI, yet a few patients don’t show the expected response³. These uncommon mutations are sensitive to first-generation EGFR-TKI in a lesser degree than common mutations.

A comprehension of the therapeutic response of various EGFR mutation to TKI is important in deciding a patient’s treatment. This encouraged the authors to observe the therapeutic response of patients with a single uncommon EGFR mutation after first-generation EGFR-TKI compared to common mutations in Indonesia.

**Methods**

Participant of this study were lung cancer patients who were treated at a tertiary hospital. Patients with stage III and IV NSCLC⁴, bearing EGFR mutation, had at least one measurable lesion (>10 mm on CT scan) were included. Patients who had incomplete initial and follow-up data, had previously received cytotoxic chemotherapy for NSCLC, had complex or TKI-resistant exon 20 T790M mutation were excluded.

This retrospective study was run from January 2016 to May 2019. The total sampling approach was done to obtain the number of participant in this study. Participants were divided into the common and uncommon mutations group. The common mutation group consisted of exon 19 deletion or exon 21 L858R and the uncommon group consisted of either exon 18 G719X, exon 18 delE790, or exon 21 L861Q.

The study procedure included collecting data from medical records of patients who received first-generation EGFR-TKI as first-line therapy. First-generation EGFR-TKIs available for use in Indonesia were Gefitinib 250 mg (Astra Zeneca Ltd, Surabaya, Indonesia) and Erlotinib 150 mg (Astellas Pharma Inc., Jakarta, Indonesia). Gefitinib or Erlotinib was taken orally, once daily. Information taken from medical records were the health-related quality of life (HRQOL), body weight, performance status (PS), and Response Evaluation Criteria in Solid Tumors (RECIST) of Chest CT. HRQOL was measured utilizing the EuroQol EQ-5D® questionnaire in Indonesian version. The questionnaire comprised of 5 simple questions, covering physical symptoms and other functional domains⁵. The EuroQol EQ-5D questionnaire in Indonesian version was declared valid and reliable to measure the HRQOL of lung cancer patients with α=80.84⁶. PS was measured by the World Health Organization (WHO) scale. Chest CT was interpreted with RECIST⁷ and the CT scan utilized was Hitachi type RH-6G-E31 series number 12G173J (Hitachi-Aloka Medical, Mitaka, Tokyo, Japan). PFS and overall survival (OS) were also observed.

The results of the study were presented in the form of mean±standard deviation (SD) or median (minimum-maximum) and percentage (%). The statistical analysis used was independent t-test or Mann Whitney test (p<0.05). Statistics analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA).

**Results**

**Characteristics of Participant**

There were more male and smoking patients in the uncommon group (Table 1). Better initial performance status was seen in in the common group. Most of the EGFR mutations in the uncommon group and common group were exon 21 L861Q (52.6%) and exon 19 deletions (64.7%; p<0.001), respectively. Most participant received Gefitinib EGFR-TKI therapy (76.9%).

**Response Evaluation in the Common and Uncommon Group**

Most patient in both groups had a constant score of HRQOL value and constant body weight after receiving EGFR-TKI therapy. Results of the CT Scan demonstrated that RECIST of most patient in the uncommon group
(47.4%) was progressive disease, while partial response was seen in most participant in the common group (42.2%; p=0.007; Table 2).

**Progression-Free Survival**

PFS could be observed in 11 and 82 participants of the uncommon and common group, respectively. The average PFS of participant common groups was longer in the uncommon group (Table 3).

**Overall Survival**

OS could be observed in 19 participant in the uncommon group and 82 out of 121 participant in the common group. The average OS of participant common groups was longer in the uncommon group (Table 3).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Gender (%)</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Smoking status (%)</strong></td>
</tr>
<tr>
<td>Non-smoker</td>
</tr>
<tr>
<td>Smoker</td>
</tr>
<tr>
<td><strong>Initial PS (%)</strong></td>
</tr>
<tr>
<td>0-1</td>
</tr>
<tr>
<td>≥2</td>
</tr>
<tr>
<td><strong>Lung cancer stage (%)</strong></td>
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<tr>
<td>IIIA</td>
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<tr>
<td>IIIB</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td><strong>Types of anatomic pathology (%)</strong></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Adenosquamous</td>
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<tr>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td><strong>Samples of anatomic pathology (%)</strong></td>
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<tr>
<td>Lung parenchym</td>
</tr>
<tr>
<td>Pleural effusion</td>
</tr>
<tr>
<td>Cervical lymph nodes</td>
</tr>
<tr>
<td><strong>Sampling technique (%)</strong></td>
</tr>
<tr>
<td>Bronchoscopy</td>
</tr>
<tr>
<td>FNAB</td>
</tr>
<tr>
<td>Core biopsy</td>
</tr>
<tr>
<td>Surgical specimen</td>
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<tr>
<td>Pleural cytology</td>
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**Cont.. Table 1. Characteristics of Participants**

<table>
<thead>
<tr>
<th>EGFR mutation (%)</th>
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<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 19 deletion</td>
<td>0 (0.0)</td>
<td>66 (64.7)</td>
<td>0.000**</td>
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<td></td>
<td></td>
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<tr>
<td>Exon 21 L858R</td>
<td>0 (0.0)</td>
<td>36 (35.3)</td>
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<td></td>
<td></td>
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<tr>
<td>Exon 21 L861Q</td>
<td>10 (52.6)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 18 G719X</td>
<td>7 (36.8)</td>
<td>0 (0.0)</td>
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<tr>
<td>Exon 18 deletion (delE709_T710insD)</td>
<td>2 (10.5)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>EGFR-TKI (%)</th>
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<tbody>
<tr>
<td>Gefitinib</td>
<td>14 (73.7)</td>
<td>79 (77.5)</td>
<td>0.769</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>5 (26.3)</td>
<td>23 (22.5)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** PS=performance status; FNAB=fine-needle aspiration biopsy; EGFR=epidermal growth factor receptor; TKI=tyrosine kinase inhibitor; *significant p<0.05; **significant p<0.001.

**Table 2. Comparison of RECIST EuroQol EQ-5D, Body weight, and PS in the Common and Uncommon Mutation Groups**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Uncommon (n=19)</th>
<th>Common (n=102)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>EuroQol EQ-5D (%)</td>
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<tr>
<td>Decrease</td>
<td>5 (26.3)</td>
<td>23 (22.5)</td>
<td>0.956</td>
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<tr>
<td>Constant</td>
<td>9 (47.4)</td>
<td>57 (55.9)</td>
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<tr>
<td>Increase</td>
<td>5 (26.3)</td>
<td>22 (21.6)</td>
<td></td>
</tr>
<tr>
<td>PS (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worsen</td>
<td>2 (10.5)</td>
<td>10 (9.8)</td>
<td>0.367</td>
</tr>
<tr>
<td>Constant</td>
<td>14 (73.7)</td>
<td>64 (62.7)</td>
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<tr>
<td>Improved</td>
<td>3 (15.8)</td>
<td>28 (27.5)</td>
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</tr>
<tr>
<td>Body weight (%)</td>
<td></td>
<td></td>
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<tr>
<td>Decrease</td>
<td>7 (36.8)</td>
<td>32 (31.4)</td>
<td>0.165</td>
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<tr>
<td>Constant</td>
<td>8 (42.1)</td>
<td>25 (24.5)</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>4 (21.1)</td>
<td>45 (44.1)</td>
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<tr>
<td>RECIST (%)</td>
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<tr>
<td>Progressive disease</td>
<td>9 (47.4)</td>
<td>26 (25.5)</td>
<td>0.007*</td>
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<tr>
<td>Stable disease</td>
<td>8 (42.1)</td>
<td>32 (31.4)</td>
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<tr>
<td>Partial response</td>
<td>2 (10.5)</td>
<td>43 (42.2)</td>
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<tr>
<td>Complete response</td>
<td>0 (0.0)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** PS=performance status; RECIST=response evaluation criteria in solid tumors; *significant p<0.05
Table 3. Comparison of Age, PFS, and OS in the Common and Uncommon Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Uncommon</th>
<th>Common</th>
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<tbody>
<tr>
<td>Age</td>
<td>56.0 (39.0-73.0)</td>
<td>55.5 (22.0-85.0)</td>
<td>0.392</td>
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<tr>
<td>PFS</td>
<td>4.0 (2.0-6.0)</td>
<td>7.0 (2.0-21.0)</td>
<td>0.001*</td>
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<tr>
<td>OS</td>
<td>4.00 ± 1.71</td>
<td>10.00 ± 6.94</td>
<td>0.000*</td>
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</tbody>
</table>

Abbreviations: PFS=progression-free survival; OS=overall survival; *significant p<0.05

Table 4. Comparison of PFS and OS in each EGFR Mutation

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
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<tbody>
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<td>PFS</td>
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<tr>
<td>Exon 21 L861Q</td>
<td>5</td>
<td>4.0 (3.0-5.0)</td>
<td></td>
</tr>
<tr>
<td>Exon 18 G719X</td>
<td>4</td>
<td>4.0 (3.0-6.0)</td>
<td></td>
</tr>
<tr>
<td>Exon 18 delE709</td>
<td>2</td>
<td>3.0 (2.0-4.0)</td>
<td>0.029*</td>
</tr>
<tr>
<td>Exon 19 deletion</td>
<td>53</td>
<td>6.0 (2.0-21.0)</td>
<td></td>
</tr>
<tr>
<td>Exon 21 L858R</td>
<td>29</td>
<td>8.0 (2.0-16.0)</td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 21 L861Q</td>
<td>10</td>
<td>4.00 ± 1.76</td>
<td></td>
</tr>
<tr>
<td>Exon 18 G719X</td>
<td>7</td>
<td>4.00 ± 1.98</td>
<td></td>
</tr>
<tr>
<td>Exon 18 delE709</td>
<td>2</td>
<td>4.50 ± 0.70</td>
<td>0.000**</td>
</tr>
<tr>
<td>Exon 19 deletion</td>
<td>47</td>
<td>11.00 ± 7.73</td>
<td></td>
</tr>
<tr>
<td>Exon 21 L858R</td>
<td>26</td>
<td>9.50 ± 5.13</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: PFS=progression-free survival; OS=overall survival; *significant p<0.05; **significant p<0.001.

Discussions

Previous studies have revealed lesser responses in uncommon mutations compared to common mutations\(^3\). In this study, most participants in the common group experienced partial response (PR), while most participant in the uncommon group experienced progressive disease (PD). The discovery that affinity of the first generation TKI to the uncommon EGFR mutation protein was lower than the affinity to common EGFR mutation of protein might play a role in this response. Up to 6-14 times higher concentrations of gefitinib are needed to inhibit the growth of cells expressing mutations G719X and L861Q, respectively when compared to cells expressing L858R\(^8\). Another comparable study found that a higher concentration of first-generation TKI was needed to cause a 50% inhibition in uncommon mutations compared to common mutations\(^9\).

In the common group, a superior response rate was seen in exon 19 deletion compared to exon 21 L858R. This finding is consistent with the results of a meta-analysis of earlier studies\(^10\). Evidence that exon 19 deletion has higher autophosphorylation rates and higher sensitivity to first-generation TKI compared to exon 21 L858R mutations\(^11\) might clarify the distinction in response rate between the two common mutations. RECIST of other uncommon mutation subtypes are dominated by progressive disease (PD), akin to the findings of previous studies where the response rate of
the uncommon mutation subtype is remarkably low\textsuperscript{10,12}. However, on the other hand, a subtype of uncommon mutation that showed a better response rate than other mutation subtypes in this study were L861Q.

Participants in the uncommon group had a shorter PFS and OS median compared to the common group. PS and smoking status are independent predictors of OS in lung cancer. In the uncommon group, the proportion of patients with good PS was less and the extent of patients with smoking history was greater than the uncommon group. This characteristics explain the shorter survival rate seen in patients with uncommon mutations\textsuperscript{13}.

HRQOL, a patient-reported outcome (PRO), was also a significant endpoint in numerous NSCLC-related studies\textsuperscript{5,14} besides response rate and survival. A large portion of the patients in both groups showed the constant EQ-5D score, indicating no HRQOL difference was found between the two groups. These conditions may be influenced by several factors, for example, employment, education, marital status, and other comorbid diseases\textsuperscript{15,16}.

In contrast to cytotoxic chemotherapy, EGFR-TKI can be given to patients with any PS with fairly good therapeutic outcomes\textsuperscript{17}. In this study, the extent of patients with initial poor PS was more noteworthy in the uncommon group than the common group. However, the evaluation of the PS of the two groups did not show significant improvement after TKI therapy. The presence of confounding variables, for example, other comorbid diseases and presence of TKI adverse effects, may likewise influence the subsequent PS. Weight loss is said to be a prognostic factor of diminished survival, decreased quality of life and more symptoms in lung cancer patients\textsuperscript{18,19}.

The limitations of this study were the small number of patients in the uncommon mutation group and the retrospective character of the study. Some baseline characteristics, such as current smoking status, duration of smoking, body mass index, presence of comorbid diseases and adverse effects of TKI, could not be fully obtained from the medical records. Further research for uncommon mutations is expected to analyze good therapeutic modalities for each subtype.

**Conclusions**

Advanced NSCLC patients with common and uncommon EGFR mutations demonstrated no significant difference in HRQOL value after receiving first-generation TKI, as observed from the EQ-5D score, PS and body weight in the two groups. However, the response rate and survival of common mutations were significantly better compared to uncommon EGFR mutations on first-generation TKI therapy.

**Ethical Approval:** Ethical approval for the research was attained at the ethics committee of hospital (1007/KEPK/III/2019).

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** None.

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Histopathological Assessment of Autopsied Salivary Gland Tissue to Estimate the Post Mortem Interval – A Cross Sectional Observational Study

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Abstract

Background: In biological system, cells are considered as dynamic and complex structures likewise cellular disintegration is also a complex process which occurs in a sequence. Therefore, these autolytic cellular changes have been investigated by forensic pathologist in an attempt to find markers that may assist in determining the time of death. In this study we attempt to analyse the efficacy of oral soft tissues specifically salivary gland in concluding the time since death.

Methodology: After obtaining approval from the human ethical committee, informed consent from the relative of the corpse and the investigating police officer, demographic information, as well as post mortem number was recorded. Submandibular Salivary Gland was collected for histopathological analysis during routine autopsy procedure. The specimens were fixed immediately in 10% formalin, processed, sectioned and stained with haematoxylin and eosin. The stained sections were evaluated under light microscope for histopathological changes.

Conclusion. The histopathological changes in the autopsied salivary gland tissue can be used as an adjuvant to estimate the post-mortem interval.

Keywords: autopsy, histopathology, oral tissue, post-mortem interval, salivary gland,

Introduction

Forensic investigation is deemed in case of person identification, to conclude the cause of death and to determine the time of death. Accomplishing the post-mortem interval (PMI) time is most challenging task in forensic investigation. There are variety of methods available to conclude the PMI such as measurement of body temperature (12-24hr), gross changes that occur after death (unreliable), (Loss of corneal reflex and changes in the eye, algor mortis, livor mortis, rigor mortis, decomposition and putrefactive changes), entomology, muscle action potential, biochemical analysis of food in stomach and intestine, electrolytes in blood, CSF, intraocular and synovial fluids, histological and histochemical study of degenerative changes in various organs and tissues, DNA quantification, analysis of organic compounds from the buried soil, radionucleotide assays. Histopathological aspect of the autopsied tissue can predict the vitality at the time of an event, the time interval since a finding was caused. In some instances histopathology can help in reconstructing an event thereby leading the investigating team, hence

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histopathology had become an integral part in forensics medicine.\(^2\) Of all the merits it is highly reliable and economic.

Pradeep GL et al., quotes that microscopically evident cellular changes in the post mortem tissues can be appreciated only after 10 hours and so they recommend tissue examination at 10hrs interval while Gururaj N and Sivapathasundharam B noticed significant cellular changes at 24 hrs interval.\(^3\) Bardale R\(^4\) observed significant changes between 6-12hrs, Yadhav AB et al., acclaim 8 hrs interval,\(^5\) However in our study we have considered the minimal time limit to record the histopathological changes i.e., 8hrs interval. There are many studies conducted on the histological analysis of labial mucosa and gingiva but studies on salivary gland is least explored specifically related to post-mortem interval and in this study we analysed the histopathological changes of salivary gland to conclude the PMI.

### Materials and Methods

The study sample comprised of autopsied submandibular salivary gland tissue (n=12) obtained from the Department of Forensic Medicine, Chengalpattu Medical College and Hospital as per Helsinki Declaration (Institutional Ethics Committee Ref No. SBDCH/IEC/10/2018/20). Only those bodies that were not subjected to refrigeration during the period between death and arrival at the mortuary and the instances of death that occurred due to road traffic accident (RTA) without any injury to face and jaws involving the study site were included in our study while all other cases including RTA with injury and presence of pathological lesion to the facial region and the study site, freezed corpse were excluded from the study.

Demographic information and post mortem number was recorded for all the corpse included in the study. Autopsied submandibular salivary gland tissues were further investigated for histopathological alterations. The acquired specimens were immediately fixed in 10% formalin, followed by routine tissue processing and sectioning, later they were stained with haematoxylin and eosin. The stained sections marked with appropriate reference number were examined under a light microscope by two independent observers, the study details were blinded. The observed histopathological changes in the acini (cytoplasm and nucleus) as well the connective tissue were categorized according to the time interval.\(^4,5\)

### Results

The submandibular salivary gland comprising of both serous and mucous acini undergo degeneration at various intervals. Initially the serous acini undergo cellular alterations followed by mucous acini. The acinar architecture is distorted in mucous acini at prolonged time interval. The various histopathological changes are shown in figure 1 and 2. The observed histopathological changes in the mucous, serous acini and the connective tissue are given in table 1.

### Table 1. Histopathological changes in post-mortem salivary gland specimen

<table>
<thead>
<tr>
<th>S.no</th>
<th>FEATURES</th>
<th>PMI &lt;8 hrs (n=10)</th>
<th>PMI 16-24 hrs (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acinar Architecture</td>
<td>Serous acini</td>
<td>Well Maintained</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucous Acini</td>
<td>Well Maintained</td>
</tr>
<tr>
<td>2</td>
<td>Loss of Cell Adhesion</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Cytoplasm Vacuolation</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Nuclear Changes: Karyolysis</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>
Cont.. Table 1. Histopathological changes in post-mortem salivary gland specimen

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Present</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Pyknosis</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Karyorrhexis</td>
<td>25%</td>
<td>100%</td>
</tr>
<tr>
<td>7</td>
<td>Nuclear Vacuolation</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>8</td>
<td>CT: Distribution Of Collagen</td>
<td>Less Homogenized</td>
<td>More Homogenized</td>
</tr>
<tr>
<td>9</td>
<td>Inflammation : Distribution</td>
<td>Diffuse</td>
<td>Diffuse</td>
</tr>
<tr>
<td>10</td>
<td>Type Of Inflammation</td>
<td>Lymphocytes</td>
<td>Lymphocytes</td>
</tr>
</tbody>
</table>

Figure 1. The acinar architecture is maintained in both serous as well as mucous acini at the initial stages but the acini exhibit cellular and nuclear changes.
Discussion

Histological assessment of autopsied tissue otherwise known as forensic histopathology clues about the vitality and the time interval of an event such as infliction of wound or aspiration of foreign material. Histopathology also concludes the cause of death,\textsuperscript{6} however the post-mortem interval assessment from the microscopic changes of oral tissues is at primitive stage.

Cellular changes in a naturally degenerating cell involves a complex sequence of events which is usually brought about by variety of enzymes. Tracking these cellular changes at post mortem may clue about many incidents that happened when the individual was alive.\textsuperscript{3}

There are few studies on post-mortem assessment of salivary gland of which one study was related to lymphocytic infiltration in labial salivary gland\textsuperscript{7} while the other was on histological assessment of the salivary gland to study the impact of hanging ligature.\textsuperscript{8} two were related to age assessment.\textsuperscript{9,10} Nery et al in 2010 studied rat sublingual salivary gland at various post mortem death intervals (0, 3, 6, 12, 24 hrs),\textsuperscript{11} Bardale R in 2013 studied human submandibular salivary gland to estimate the time since death.

In our study sample of mixed salivary gland (submandibular salivary gland), though there were remarkable cytoplasmic and nuclear changes in both serous and mucous acini they were not so critical to consider as a predictor for post-mortem interval assessment. However, the architectural changes are highly promising, where only the serous acini undergoes degenerative changes at the earlier stage itself. Loss of cell adhesion and cell wall disruption leads to clumping of nucleus to the centre of the acini both serous and mucous forming a filigree pattern in the later stage that is between 16-24hrs. Our study results are consistent with that of Bardale R and Nery et al.,\textsuperscript{4,11} where they have also reported that serous acini are faster to undergo

Figure 2. The acinar architecture is distorted in serous and mucous acini at the later stages, also there is homogenization of collagen fibres.
autolysis while mucous acini undergo degenerative changes close to 24 hrs.

**Conclusion**

Through this study, it is evident that histopathological changes in mixed salivary gland can aid in predicting post-mortem interval. Though there is environmental influence on the autolysis, different oral tissues from the same person have to be evaluated for exactness of the timing. Also there are very little scientific evidence regarding the utility of microscopic aspect of oral tissues in the prediction of post-mortem interval. The gap must be over-ruled by including forensic dentist and oral pathologist in routine autopsy procedure.

**Ethical Clearance** – Obtained from Chengalpattu Medical College and from Sree Balaji Dental College.

**Source of Funding** - Self

**Conflict of interest** - NIL

**References**


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Influence of Melatonin in the Treatment of Experimental Enterobius Vermicularis Infection

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¹Professor, Pharmacy College/Kufa University, ²Research Scholar / Pharmacy College / Al-kafeel University, ³Research Scholar / Dentistry College/ Al-kafeel University

Abstract

This study aims to realization the conceivable therapeutic of melatonin effects experimental against Enterobius vermicularis in rats. Implement this experiment during the period from August 2019 to January 2020. E.vermicularis infected with male wistar rats orally with dose 15mg/kg melatonin former of body weight for 30 day showed significantly reduction in the number of eggs and worms compared with rats orally with dose 15mg/kg melatonin accompanying and untreated rats for 30 day (P < 0.05).Histologically in intestine examined show increase numbers of leucocytes produce, necrosis significant scatter and reduction this parasite of tissue in rats treated with melatonin. This results show influence of melatonin in the control on Enterobiosis and suggestion that this drug usefulness in Enterobius vermicularis infection therapy.

Keyword: Eggs, Worms, Enterobius vermicularis, Melatonin, Former.

Introduction

Enterobius vermicularis is helminthes more common human parasitic Nematoda infected the bowel but the children worldwide may reach to 40 million infestations in USA and Europe especially school students (¹). Infection may be associated with poor hygiene or behavioral environments in family overcrowded and orphanages where transfer the eggs pinworm from person to another by finger polluted or via anus into mouth directly may transmit by eat contaminated food indirectly (²). The clinical symptoms occurs because the migration of the gravid female worst at night when lays eggs lead to excitement, lack sleep, appetite and weight decrease , vomiting and abdominal pain (³).

There are many drugs can be help in eliminated on pinworm else will not be beneficial, most common drug is mebendazole family these killed the adult worms only addition to increased resistance these drugs wherefore need for the development of new methods for control and enucleate of the parasitic disease (⁴).

Recently studies suggests that melatonin immune enhance function through presence of melatonin receptor in immune organs, Melatonin is biological processes recurring naturally hormone synthesized in most the pineal gland to blood of mammals also is synthesis in deferent cells, tissues and organs like lymphocytes, skin, eyes and gastrointestinal duct (⁵). Melatonin has been examination studies in parasitic, virus and bacterial infestations (⁶). Act the melatonin to promote antigen display, phagocytic activities and production of monocytes (⁷). Melatonin have important immune-modulatory effects e.g. Plasmodium that hepatocytes colonies and red blood cells will causes in death of malaria through that melatonin have precursors derived from the tryptophan will calcium release and modulate the cell cycle of P. falciparum (⁸). The melatonin treatment with Schistosoma mansoni act on decrease oxidative injury and increase permanence of hamster infected (⁹).
The goal of this study to specify influence of melatonin drug against *Enterobius vermicularis* by examined in rats.

**Materials and Methods**

**Collection eggs of *Enterobius vermicularis***:

Eggs were collected from infected children of school in Al-Najaf city, gathered in anus they suffer from anal itching by transparent adhesive tape (10), these eggs incubation at 36°C in wet flask for 5 days, most eggs were ivied released through vexation of the body, these eggs contained within larva notice circulation movement after three from incubated inside shell, some of them hatch naturally as expressed (11) kept unit used in the experiment.

**Preparation of Melatonin Solution**

Consider melatonin slightly soluble in water so used dimethyl sulfide and ethanol (DMSO/Germany) to dissolve. Take 2 mg / milliliter 99.9 DMSO-melatonin were prepared as stock solution (12).

**Preparation and Infection Animals**

90 male wistar rats were weight 100-110 g kept under light period 12h light and 12h dark where divided into three groups each group contain 30 rats were placed in plastic cages contain food and water with a floor furnished with sawdust, good ventilation and continuous cleaning of the cages,500 eggs number within movement larva examined under microscopic were counted from eggs sedimentation by slide chamber, group one were 30 rats infected with 500 egg of *E.vermicularis* only orally as control without drug, group two (Former) were 30 rats were inoculated melatonin pretreated for 7 days before the infection daily orally at dose of 15 mg / kg body weight where dissolved in distilled water then give oral 500 eggs for 30 day and group three (Accompanying) inoculated melatonin with eggs daily at dose of 15 mg / kg body weight give oral 500 eggs for 30 days. Three groups were examining the stool after 10, 20 and 30 days of infection by microscope.

**Histology Animals**

Rats were numbness with 2.5 pentobarbital and postmortem, intestine were reapers then inglorious 10 formaldehyde to make a histological section of the infection and treated, eosin-haematoxylin stain then examined by microscope in magnification of 100x (13).

**Statistical analysis**

Results were calculated by analyses data the one way by ANOVA test and statistical significance between groups analyses when (P < 0.05).

**Results**

As shown in table (1) , there is a significant reduce in *E.vermicularis* infection in rats treated with melatonin former at the dose of 15 mg/kg which were 1 and 0 for eggs and worms respectively, while there is a significant decrease in *E. vermicularis* infection in rats treated with melatonin accompanying at a dose of 15 mg/kg which were 21 and 10 for eggs and worms respectively, both after 30 day of treatment compared with control without treated were 390 and 495 for eggs and worms respectively after 30 day of infection. This may due to protective effect of melatonin is put off the appearance of disease, retard death and reduce the mortality rate.

**Table 1: Influence of Melatonin drug on count of Eggs & Worms of *Enterobius vermicularis* in rats per 20 microscope fields / days.**

<table>
<thead>
<tr>
<th>Dose Mg/kg</th>
<th>10days</th>
<th>20days</th>
<th>30days</th>
<th>F</th>
<th>P value</th>
<th>LSD = 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>500</td>
<td>473</td>
<td>470</td>
<td>480</td>
<td>390</td>
<td>495</td>
</tr>
<tr>
<td>Melatonin Former</td>
<td>213</td>
<td>92</td>
<td>45</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>15 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melatonin Accompanying</td>
<td>322</td>
<td>211</td>
<td>105</td>
<td>57</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>15 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LSD : Least Significant Difference

Discussion

Enterobiosis is a human intestinal parasitic disease caused by pinworm infects a lot of people especially children causes symptoms e.g. anal itching, painful or difficult urination, irritation, insomnia repeated infection causes weakened immunity and may lead to death in the absence of treatment (14). Because of resistance to conventional drug and repeated infection, must search for alternative drugs and low toxicity (15). In the present study used melatonin drug is suggested that can therapeutic differ agent like immune enhance functions, antioxidant effect, bacterial, fungi viral, and parasites infections, shown significant reduce E. vermicularis with melatonin former when dose 15 mg/kg were 1 and 0 for eggs and worms respectively while significant decrease E. vermicularis with melatonin accompanying when dose 15 mg/kg were 21 and 10 for eggs and worms respectively, both after 30 day of treatment compared with control without treated were 390 and 495 for eggs and worms respectively after 30 day of infection, this indicates that give melatonin former enhances of the immune response, as in Table 1.

This may due to protective effect of melatonin is put off the appearance of disease, retard death and reduce the mortality rate (16), these study consistent with (17) that melatonin have control through of experimental the Trypanosoma cruzi infection and lead to reduce the parasitemia levels in rats. Another reported by (18) that melatonin drug cellular immunity activity by increased production lymphocyte in Toxoplasma gondii infected in rats. As in other study show reduce Leishmania infection to 40 in hamsters infected during the when serum melatonin being high compare to animals infected when melatonin level being low, this indicates that melatonin receptors plays an important role in leishmaniases treatment (19).

As shown in the current study, it have been seen in histological analysis for untreated section granuloma fashioning in the intestine, necrosis, adenoma and hemorrhage of the bowel (20).

There was a statistically increased numbers of leucocytes production which observed in both the accompanying and former melatonin treatment observation tissues necrosis scatter among regions and inflammatory cells sneak shrill comparison with E. vermicularis infection only (P < 0.05) may due to melatonin increased immune-modulatory activates and have ability on stimulate innate immune cells in positive attachment between melatonin and phagocytic efficacy with infected (21).

This study agreed with (22) showed that exogenously manage melatonin significant reduced the amoebic necrosis areas also increased of leukophagocytosis and number of the dead amoebae.

In other study Trypanosoma brucei parasite was given the melatonin infected rats make histological changes in pineal gland where caused in reduce plasma level which may due to release of inflammatory mediators and become not inroad cell (23).

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

Conflict of Interest : Nil

References


The Observational Study of Reduction in Inflammatory Markers and Simultaneous Reduction in Joint Inflammation in Patients of Rheumatoid Arthritis Treated by Leech Therapy

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Abstract

The sense of pain in arthritis carries the highest stigma among the sufferers as well as the treating physicians. It makes the life miserable for the patients along with the disability the disease rheumatoid arthritis causes, which can be correlated to Aamvata in Ayurveda. There are the conventional treatment options which are available in the form of shodhana and shanmana. Besides NSAIDs and corticosteroids in modern medicine rendering many side effects Ayurveda offers drugless healing art in the form of jaloukavacharan also known as leech therapy which is known for the anti-inflammatory and analgesic, anesthetic actions of leech salivary gland secretions. Hence, a protocol was designed for the doctoral research on “Study of inflammatory markers in patients of Rheumatoid Arthritis treated by leech therapy”, for the assessment of subjective parameters of pain, swelling, redness, tenderness, loss of function of joints assessed by standard criterion, and objective parameters like CRP and ESR before and after leech therapy for 10 sittings on alternate days. Observations were taken on day 0 and day 21, and follow up taken on day 30 and day 45. Statistical tests were applied which revealed significant results of anti-inflammatory effect of leech therapy on CRP (relative change 23.54%), with p value =0.0001, than ESR (relative change 10.30). Leech therapy was found to be having highly significant effect on pain score (relative change 57.62%), followed by tenderness score (relative change 72%) and followed by significant increase in walking effect (relative change 56.67%). There was statistically significant improvement in other subjective parameters of assessment like swelling on ankle (relative change by 11.73%), knee joint (relative change 10.29), redness (relative change 25%), walking effect (relative change 72%), rise in local temperature (relative change 61.90%) with p value 0.0001 with co-relatable clinical improvement.

Keywords- Leech Therapy, Inflammatory markers, Aamvata, Rheumatoid arthritis, analgesic, anesthetic

Introduction

The aim of all Medical Sciences is to provide better health to every human being so as to have a disease free life. With this aim the Indian System of Medicines with respective doctrines are trying their best for one goal i.e. “Conservation of Health by Natural Methods using Nature”. World Health organization is also trying it’s best to integrate all Medical systems to achieve the goal of “health for all”. Ayurveda medical science with its holistic approach has to play a vital role in this direction. “That’s the ideal medicine, which relieves from all kinds of miseries,” says Charaka.¹ Drug, is not necessarily be given orally. Ayurveda speaks about many modes of healing art, even surgery and para-surgical techniques. Among the para-surgical measures, Raktamokshana enjoys a place of pride since the dawn of medical history. Raktamokshana is a technical term employed to denote the para-surgical procedures to expel out the vitiated blood from selected areas of the body, by specific methods.²

The prevalence of RA is between 0.5 and 1% of adults in the developed world with between 5 and 50 per 100,000 people newly developing the condition each year.³ Of the variety of researches done in arthritis, most of them are done on subjective parameters like pain, swelling, tenderness, etc but not on objective and
specific parameters like Inflammatory markers (CRP and ESR) which carries a point value in ACR/EULAR scale. The ultimate goal of treatment is to reduce pain, decrease inflammation, and improve a person’s overall functioning but associated with them are the systemic side effects and prolonged use is not recommended. New classification criteria overruled the “old” ACR criteria of 1987 and are adapted for early RA diagnosis. The “new” classification criteria, jointly published by the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) establish a point value between 0 and 10. In these, of the 10 points, 1 point is attributed to elevated ESR (erythrocyte sedimentation rate), and or elevated CRP value (C-reactive protein).5

Aetna Journal considers medicinal leech therapy experimental and investigational for treating cancer pain, epidermoid cysts, knee osteoarthritis, inadequate arterial supply or tissue ischemia, priapism, rheumatoid arthritis and other musculoskeletal diseases, and for all other indications because of insufficient evidence of its effectiveness.6

**Research gap analysis** - All the studies done till now are with some Ayurveda pharmacological intervention and Leech therapy and based on subjective parameters like Pain, Swelling etc. less emphasis was given on the objective parameters like Markers of Inflammation (CRP and ESR)

**Research question** - Can the study of inflammatory markers in patients of Rheumatoid Arthritis may be of utility to ascertain efficacy of Leech Therapy with reference to pain, inflammation and swelling?

**Aim** - Assessment of the efficacy of Leech Therapy in the patients of Aamvata (Rheumatoid Arthritis) by evaluation of reduction in Inflammatory Markers(CRP and ESR)

**Objectives** - 1) Pre and Post treatment evaluation of inflammation, CRP and ESR in RA patients.

2) To compare the anti-inflammatory effect(Pain and Swelling) of Leech Therapy Pre and Post treatment in patients of Rheumatoid Arthritis (wsr. Aamvata)

3) To compare the levels of CRP and ESR after leech therapy in RA patients

**Anticipated translator component**

1. A more easily available natural mode of management of RA (Aamvata) in the form of leech Therapy may be introduced in routine practice.

2. If the hypothesis is proven and Leech therapy is found to be effective in the reduction of levels of CRP and ESR , a non-pharmacological method which is used as Biotherapy may be introduced in reducing the markers of inflammation in patients of RA.

3. Similarly, leech therapy is a known para-surgical tool in the treatment of some peculiar features of arthritis like joint pain and restricted movement, hence, it can be validated in this study on RA, for re-establishment.

**Research Design:**

Nature of study: Experimental Study.

Study type: Open label single group experimental study

Commensurate Sample Size: Total 61 patients were selected, allocated into a single group by convenience sampling method.

**Variables** – CRP (evaluated by quantitative method), ESR (evaluated by quantitative method), Pain (VAS scale), Swelling (Metric method), Redness (present/Absent), Tenderness (Dr. Frank Painter’s scale), Rise in local temperature (Present/absent) were the variables and criteria of assessment in the patients of RA.

**Review of literature** - Amavata is one of the crippling diseases, claiming the maximum loss of human power. It is not only a disorder of locomotor system but is a systemic disease and is named after its chief pathogenic constituents i.e. Ama and Vata. Sandhishoola, Sandhishotha, Stabdhata and Sparshasahyata are salient features of the disease. The disease Amavata run a chronic course and Jadya, Sankocha, Anga vaikalya etc. are responsible for crippling of the patients in the long run.7

This disease can be correlated to Rheumatoid Arthritis that stands parallel to Amavata in its clinical features. RA is a chronic inflammatory arthropathy, which most commonly affects middle aged women. Despite intensive research, the aetiology of Rheumatoid Arthritis remains unknown. In spite of available
treatment options, it cripples the patient for the rest of his life. Moreover, it affects the younger and middle aged people, substantially hampering the economy and affecting the productivity of the society and subsequently of the Nation. Thus, the disease has great challenge to the Clinicians and Researchers.8

According to the symptomatology Amavata is very similar to rheumatoid arthritis, a disease of unknown aetiology. So many hypotheses have been put forward to explain its aetiology but still the research is going on. Nowadays theories of autoimmune mechanism, genetic susceptibility and free radicals are most commonly incriminated role in etiopathology of Rheumatoid Arthritis. The diagnosis is made mostly on the basis of a person’s signs and symptoms. X-rays and laboratory testing may support a diagnosis or exclude other diseases with similar symptoms.9

**Inflammatory markers** - The Erythrocyte sedimentation Rate (ESR) and C-reactive Protein (CRP) are blood tests that are Markers to detect Inflammation. These are certain useful blood tests to help diagnose and monitor the inflammatory activity and response to treatment in diseases like Rheumatoid Arthritis, diabetes, Alzheimer’s disease, Osteo Arthritis, Cellulitis, SLE, etc. The “new” classification criteria, jointly published by the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) in 2012 and 2019 establish a point value scale between 0 and 10 to diagnose RA based on the levels of ESR and CRP. In these, 1 point is attributed to elevated ESR (erythrocyte sedimentation rate) or elevated CRP value (C-reactive protein).10

**Leech Therapy** can be defined as the use of leeches in medical treatment. This therapy helps in letting out impure or deoxygenated blood from given area with the help of leech bite. The Leech sucks blood from the site as well as transmits some enzymes in its saliva having anesthetic, anti-inflammatory, anticoagulant, vasodilatation effect etc. thereby giving prolonged oozing effect from the site of bite. Studies suggest that leech SGS (Salivary gland secretions) contains more than 150 bioactive substances and has anti-edematous, bacteriostatic, analgesic, resolving actions. It eliminates microcirculation disorders, restores damaged vascular permeability of tissues and organs, eliminates hypoxia, reduces blood pressure, increases immune system activity, detoxifies the tissue, releases it from the threatening complications, such as infarct, stroke, improves bio-energetic status of the organism through rejuvenation. Hence Leech Therapy has been established as one of the most efficacious therapies in Ayurveda but due to lack of relevant evidence not in vogue.11

**Materials** - Leeches and Leech Lab and other procurements as per the SOPs of Jaloukavacharana12 and tablets of Paracetamol

**Leeches and leech lab**

![Leeches and leech lab](image1.jpg)

Pic no-1,2,3 Leech lab, labeled containers for patients and Required material for Leech therapy

Photos of patients with RA on knee joints

![Photos of patients with RA on knee joints](image2.jpg)

Pics no 4,5,6,7
Photos of patients with RA on ankle joints

Pics no 8,9,10,11

Inclusion Criteria—Patients in the age group 20 to 70 yrs. with special features of RA were selected for study like two or more swollen joints and with elevated erythrocyte sedimentation rate (ESR), and or elevated CRP value (C-reactive protein) were selected for the research protocol.

Exclusion Criteria—All infective types of Arthritis, pregnant ladies, lactating mothers, HIV and Hbsag positive patients, patients on treatment of IHD, patients with bleeding disorders.

Methods—The patients suffering from Aamvata (RA) in whom knee joints were involved is the target population.

Patients of Aamvata, whether sero positive or sero negative, in whom CRP and ESR were raised were chosen for leech therapy. Painful Knee joint and ankle joint (were preferred for Leech therapy). Leech Therapy was done on alternate day. Such 10 sessions were done. On every instance, 2 or 3 leeches (small to moderate size were applied which were found to suck 5 to 15 ml of blood as average.

This is a single group interventional Study in which pre and post treatment assessment was done on certain objective parameters like markers of inflammation i.e. CRP and ESR and subjective parameters like pain, swelling, redness, tenderness, rise in local temperature, restricted movements of joints. Blood investigations like CBC, CRP, ESR, RA factor, Bleeding time and Clotting time, HIV and Hbsag were performed prior to leech therapy. CRP, ESR, BT and CT were repeated after leech therapy.

The observations were taken on day 0 and day 21 of leech therapy. Leech Therapy was performed on alternate day on affected joint according to the standard operating procedures of Raktramokshana by Jalouka, on preferably the knee and ankle. The observations were compared before and after therapy and statistical data drawn quantitatively.

Results and Discussion

In the doctoral research “Study of inflammatory markers in patients of rheumatoid Arthritis treated by leech therapy”. the sample size was 61 calculated as a convenience sampling with simple randomization. The distribution of patients according to various parameters was done. Statistical tests were applied and data is drawn which is described in tables.

Table no 1- Distribution of patients according to gender-

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>28</td>
<td>45.9%</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>54.1%</td>
</tr>
</tbody>
</table>

Table no. 1 reveals that, of the 61 patients, 28 were male and 33 were female.

Table no 2- Distribution according to Chronicity of Symptoms

<table>
<thead>
<tr>
<th>Duration (months)</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 6</td>
<td>16</td>
<td>26.23</td>
</tr>
<tr>
<td>7 to 24</td>
<td>28</td>
<td>45.9</td>
</tr>
<tr>
<td>More than 24</td>
<td>17</td>
<td>27.87</td>
</tr>
</tbody>
</table>
Table no 2, reveals that, when 3 groups were formed to classify the chronicity like 0 to 6 months, 7 to 24 months and more than 24 months, maximum no of patients ie 28 were found in 7 to 24 months group.

**Table no 3-Distribution of subjects according to Type of Pain**

<table>
<thead>
<tr>
<th>Type of pain</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bheda(Throbbing)</td>
<td>13</td>
<td>21.31</td>
</tr>
<tr>
<td>Daha(Burning)</td>
<td>13</td>
<td>21.31</td>
</tr>
<tr>
<td>Kartanvata(cutting)</td>
<td>11</td>
<td>18.03</td>
</tr>
<tr>
<td>Toda(pricking)</td>
<td>13</td>
<td>21.31</td>
</tr>
<tr>
<td>Vruschik danshvata</td>
<td>11</td>
<td>18.03</td>
</tr>
</tbody>
</table>

According to the distribution of type of pain, Table no 3, the type of pain on highest frequency was toda, Bheda and dha ie13 patients each were found to suffer from pricking, throbbing and burning type of pain.

These were the demographic criterion illustrated in n-61.

**Table no 4 -Summary table showing effect of leech therapy on important parameters**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Before</th>
<th>After</th>
<th>Absolute Change (After-Before)</th>
<th>Relative (% change from baseline)</th>
<th>Significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain (%) (moderate+severe)</td>
<td>100</td>
<td>60.78</td>
<td>-39.22</td>
<td>-39.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pain score (mean±SD)</td>
<td>75.77</td>
<td>32.11</td>
<td>-43.66</td>
<td>-57.62</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ankle Swelling (mean±SD score)</td>
<td>32.82</td>
<td>28.97</td>
<td>-3.85</td>
<td>-11.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>Knee Swelling (mean±SD score)</td>
<td>37.21</td>
<td>33.38</td>
<td>-3.83</td>
<td>-10.29</td>
<td>0.0001</td>
</tr>
<tr>
<td>Tenderness (%)</td>
<td>91.67</td>
<td>35</td>
<td>-56.67</td>
<td>-61.82</td>
<td>0.0001</td>
</tr>
<tr>
<td>Redness (%)</td>
<td>81.67</td>
<td>56.67</td>
<td>-25.00</td>
<td>-30.61</td>
<td>0.0079</td>
</tr>
<tr>
<td>Distance walked (mean±SD meters)</td>
<td>16.25</td>
<td>27.96</td>
<td>+11.71</td>
<td>+72.06</td>
<td>0.0001</td>
</tr>
<tr>
<td>Range of motion (mean±SD degrees)</td>
<td>45.64</td>
<td>88.61</td>
<td>+42.97</td>
<td>+94.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rise in local temp (%)</td>
<td>91.87</td>
<td>35</td>
<td>-56.87</td>
<td>-61.90</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Results-The table no 4 above reflects the overall effect of leech therapy on the clinical assessment parameters like pain, swelling, redness, tenderness, rise in local temperature, restricted movement and walking effect. The effect of therapy was also assessed on pathological parameters like inflammatory markers CRP and ESR at the end of 10 leech sessions on alternate days. The overall effect was assessed on day 21 of therapy, that shows significant effect on all the important parameters statistically with p values =0.0001 which is highly significant. Maximum relative change was found in walking effect in the patients of RA which is found to be 72.06%. The relative change in pain score is found to be 39.22%. In Swelling it was found to be 57.62% in knee and 11.73% in ankles. The relative change in tenderness and redness on joints are found to be 61.82% and 30.61% respectively. The relative change in range of motion is 94.15% and in rise in local temperature, it is found to be 61.90%.

Discussion-These findings are the outcome of the anti-inflammatory and analgesic effect of enzymes in leech salivary gland secretions like bdellins, eglins, factor Xa inhibitor, protease inhibitors, tryptase inhibitors, Cathepsin G, histamine like substance, Hyaluronidase, carboxypeptidase inhibitors, acetyl choline, and Anesthetic substance, and anti-coagulant substance called Hirudin and hirustasin which cumulatively give the relative change effects of reduction in pain, swelling, redness, tenderness, rise in local temperature, and improved walking effect stated as above as p=0.0001 level of significance. The combined effect of the multiple bioactive salivary secretions like pain killer action, analgesic anti-inflammatory action, anesthetic properties, thrombolytic effect, tissue rejuvenation effect, anti-ischemic effect, renders the improvement in the pain, swelling, stiffness of the joint and improvement in the restricted movement of joint13.

As Leech therapy is effective in giving pain relief, reducing swelling, redness, tenderness and local warmth is also reduced improving the restricted movement of joints of lower limb. Singh et al state that all the improved characters play a combined role on the walking effect of the patient thereby improving the quality of life of the patient.

Results-The relative change in CRP after leech therapy is found to be 23.54% which is found to be highly significant. The relative change in ESR levels is found to be 10.30% which is statistically somewhat less significant.

Discussion-These findings can be justified by the fact that Overall anti-inflammatory effect of leech therapy by the virtue of various enzymes in its salivary gland secretions gives the reducing effect on inflammatory markers.14 Besides, the difference in the rate of reduction of both CRP and ESR can be justified by the fact that plasma half-life of ESR is up to certain weeks to 2 to 3 months, while that of CRP is 19 hours. Hence for plasma ESR levels to reduce, takes a long span of time which may be in months, because even if the disease process is over ESR is found to be high with other confounders in coexistence. CRP on the other hand is a protein which increases when the inflammatory process is activated in the body with peak plasma levels for 19 hours and regression later. Hence it is the rate of synthesis which
determines the response to treatment. Hence after 10 sittings of leech therapy, if CRP levels are deterred by 23.54% then it is found to be a significant difference with arrest of inflammatory process at a certain level. CRP being a specific marker of inflammation, is a significant finding as a regressed marker after treatment with leech therapy.

Limitations of the present study-

As the effect of leech therapy on CRP and ESR could be found to render statistically significant but parametrically less significant results, it can be used as an adjuvant therapy in the treatment of Aamvata.

Immediate post-doctoral Research direction & Suggested future studies-

Ø This study should be done on large population.
Ø Multicentric, Interdisciplinary research with RCT design should be conducted.

Achievable translatory component:

Ø Many patients suffering from Rheumatoid Arthritis(Aamvata) having inflammatory arthritis and painful joints along with features of stiffness and inability to walk can be effectively treated with Leech therapy on Joints.
Ø CRP is a marker of inflammation which is significantly attainable by treatment with leech therapy.

Declaration of new knowledge generated:

Ø Leech Therapy is significantly effective in decreasing the CRP to a significant level and ESR to considerable levels and effectively renders pain relief to the patients of Rheumatoid Arthritis.

Ethical Clearance- Taken from Institutional ethics committee, JNMC, Saongi, Wardha, in 2016

Source of Funding- Self

Conflict of Interest - Nil.

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Comparative Study of *Palashgudavarti* and Diclofenac Sodium Suppository in the Management of Acute Fissure in Ano (Parikartika)-An Ayurvedic Management Protocol

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Abstract

Background - Anal Fissure is one of the major causes for pain at anal region. The two primary signs of this disease are pain and bleeding, and pain is often unbearable. In males anal fissure typically occurs in the midline posterior- 90 %, and 10 % midline anterior. Subsequently, female fissures on the midline anteriorly are significantly more frequent than posteriorly (60:40). In contemporary sciences *Parikartika* can be correlated with Fissure in Ano. Objective- The objective of the prospective study is to find the efficacy of *Palashgudavarti* & Diclofenac Sodium Suppository in the management of Acute Fissure in ano (*Parikartika*). Material & Method: The present study is designed as a Randomized single blind parallel in which 60 patients will be enrolled. Varti will be applied for local application once a day. Assessment will be done 0th, 7th day, 14th day, and 28th day. Results: The changes are expected to be observed in subjective parameters such as pain bleeding per rectum with itching as well as with objective parameters such as *Parikartika* Healing. Conclusion: The study is expecting the nonsurgical management of fissure in ano with respect to the impact of *Palashgudavarti* & Diclofenac Sodium Suppository. The research is expecting to be baseline and benchmark of the prospective studies in Acute Fissure in ano (*Parikartika*).

Keywords: *Parikartika*, fissure in ano, *Palashgudavarti*

Introduction

Lifestyle disorders are one of the most common condition seen in society. Lifestyle diseases are mainly caused by improper work pattern, stressful life, improper diet intake and improper sleep habits. These causative factors produce indigestion which leads to various lifestyle disorders specially anorectal disorders like piles, fissure in ano, fistula in ano etc. constitute a significant group. Among all anorectal disorders, fissure in ano is the most common disease. Anal Fissure is one of the major causes for pain at anal region The fissure-in-ano is categorized into two types depending on the clinical symptoms & durations of the disease that is Acute and Chronic fissure in ano. The two primary signs of this disorder are, bleeding and pain; pain is often unbearable. In chronic conditions, sentinel tag and haemorrhoids can be associated with this. Pruritus ani can be present sometimes with this disorder¹. In males anal fissure typically occurs in the midline posterior- 90 percent and 10 percent much less frequently. Subsequently, female fissures on the anterior midline are somewhat more common than before. (60:40)²

*Parikartika* can be correlated with Fissure in ano in modern medical sciences. There are different causative factors of *Parikartika* such as *Vamana–Virecana Vypada*, Basti Karma vypada and *Upadrava* of *Atisara*, *Grahani*, *Arśa*, *Udāvarta*³. In this regard Acharya *Sushruta* explained the pathogenesis of disease⁴

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The word Parikartika means Parikartanavatvedana around guda i.e. cutting type of pain. Parikartika also has symptoms such as pain in the penis, anus, neck of the urinary bladder and umbilical region with flatus cessation. References about Parikartika are available from all Bruhatrayi and also mentioned in kashyap samhita and later authors of Ayurveda.

Methodology

Trial design: Randomized single blind parallel.

Study setting: Diagnosed Patients will be selected from Shalyatantra OPD & IPD of M.G.A.C.H. and R.C. Wardha.

Figure 1 Flow diagram of the study procedure

CTRI NO- CTRI/2020/09/027734

IEC No- MGACHRC/IEC/August-2020/97

Inclusion Criteria:

- Patients willing for the consent
- Patients with age group of 20 yrs to 50 yrs
- Patients with clinical features of Acute Fissure in ano will be included after screening.
- Patients irrespective of gender, occupation and economic status will be included

Exclusion Criterion:

- Patients suffering with systemic disorders like Diabetes mellitus, Tuberculosis, HIV Positive, and Hepatitis B.
- Known cases of Malignancy, Crohn’s disease, Ulcerative colitis will be excluded.
- Chronic patient with 4th grade anal spasm will be excluded.

Criteria for discontinuing or modifying allocated interventions: Patients will be withdrawal from intervention if any harmful incidence, signs of drug allergy or any problem will occur; patient will be offered treatment free of cost till the disease subsided.

Follow up period after treatment: 28th day after treatment.

Primary Outcomes: Changes in the symptoms of Fissure such as Pain, Bleeding, Itching.

Secondary Outcomes: To observe the changes Healing

Statistical analysis: Wilcoxon rank sum test.

Time duration till follow up: Follow up days is 0th, 7th, 14th, 28th days.

Time schedule of enrolment, interventions: Diagnosed patients of Fissure in Ano will be enrolled in the present study after fulfilling the inclusion criteria.

Interventions- One Varti of 2gm will be taken for local application after Hot Sitz Bath once a day during the treatment period.

Groups - Two groups with 30 patients in each.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size</th>
<th>Local Application of Ghrita</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>30</td>
<td>Palashgudavarti</td>
</tr>
<tr>
<td>Group B</td>
<td>30</td>
<td>Diclofenac Sodium Suppository</td>
</tr>
</tbody>
</table>

Recruitment: Patient will be recruited by single arm study

Implementation: Principal invigilator will register subject.

Data collection methods: Randomized

Assessment criteria:
a) Subjective criteria

1. Pain - Vas Scale
2. Bleeding per Rectum – Truncated rectal score for rectal bleeding
3. Itching – Numerical Rating Scale

b) Objective criteria

1. Parikartika healing – Southampton wound scoring system

Data management: Principal investigator will do coding of data.

Ethics and dissemination: Permission for research has been taken from Institutional Ethical Committee ref no. IEC No: MGACHRC/IEC/August-2020/97

Consent or assent: Written informed consent will be obtained from the patient.

Dissemination policy: For future research results will be disseminated and research will be published in reputed journal

Informed consent materials: All the research related document and consent form will be given to the patients.

Figure no. 1 Gnatt Chart (Quarterly based)

<table>
<thead>
<tr>
<th>Scholar/Investigator</th>
<th>Dr. Shubham Biswas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>“Efficacy of Palashgudavarti in comparison with Diclofenac Sodium Suppository in the management of Acute Fissure in ano (Parikartika)”</td>
</tr>
<tr>
<td>Steps</td>
<td>Q1</td>
</tr>
<tr>
<td>Enrolment of Patients</td>
<td></td>
</tr>
<tr>
<td>Drug Collection and preparation</td>
<td></td>
</tr>
<tr>
<td>Data Collection</td>
<td></td>
</tr>
<tr>
<td>Writing thesis parts up to Methods</td>
<td></td>
</tr>
<tr>
<td>Data Analysis</td>
<td></td>
</tr>
<tr>
<td>Writing rest of thesis</td>
<td></td>
</tr>
<tr>
<td>Submission</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In Ayurveda, palash is used for the treatment of vrana, gulma, bhagnasandhana, grahani, arsha, gudaroga\(^7\). Rasapanchak of Chakramarda is Rasa-Katu, tikta, kashaya, Virya-Ushan, Vipaka-Katu, Karma-Vata-Kaphahara\(^8\). Palash has anti-inflammatory activity, analgesic, antifungal activity and anti-ulcer activity\(^9\) It is known for Vranaropak properties. Fissure in ano is longitudinal tear in the anoderm distal of anal canal. In contemporary sciences surgical treatment available for fissure in ano are sphincterotomy, lord’s dilatation, fissurectomy, but these surgical procedures having adverse effects such as bleeding, infection, incontinence\(^10\). To sort out these problem we need treatment which is easily applicable and non surgical
Expected result and conclusion

Palash has anti-inflammatory activity, analgesic, antifungal activity and anti-ulcer activity. The predicted outcome of this analysis is that group A with intervention is more effective intervention to group B. It is effective in subsiding the symptom of *parikartika* such as pain & bleeding per rectum, itching. Patients who take all follow-up after treatment will have less chance of symptom reoccurrence.

References
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Review on *Annavahasrotodushti* with Respect to *Arsha Vyadhi*

Shubham Golokkumar Biswas¹, Devyani Dasar², Alok Kumar Diwedi²

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Abstract

Digestion is a main process of human body. Food is essential for body and digestion, conversion of food takes place in digestive tract. *Annavaha Srotas Dushti* leads to indigestion, anorexia, lack of interest towards food which leads to Anorectic disorders like *Arsha, Parikartika* etc. Contrarily this seems to be an increasing incidence of the commonest of all anal disease, i.e. Haemorrhoids and fissure in ano. Though *Sushruta* has described detailed of, however as lifestyle has been changed significantly therefore it is of great importance to study other causes of Haemorrhoids according to modern era. Hence, the diseases of ano-rectal area are the commonest of all the diseases of GI tract. This study aimed at *Annavahsroto-dushti* and its relation in Pathogenesis of *Arsha Vyadhi* and its management. They were well known for time immemorial to the physician and surgeon due to the worldwide distribution and trouble caused by them.

**Keywords**- Annvahasrotas, Anorectal, Arsha.

Introduction

Digestion is a main process of human body. Food is essential for body and digestion, conversion of food takes place in digestive tract. *Annavaha Srotas* is the channel for transportation, digestion and absorption of food. *Annavaha Srotas Dushti* leads to indigestion, anorexia, lack of interest towards food which leads to constipation and may results in Anorectic disorders like *Arsha, Parikartika* etc. Contrarily this seems to be an increasing incidence of the commonest of all anal disease. Though *Sushruta* has described detailed of, however as lifestyle has been changed significantly therefore it is of great importance to study other causes of Haemorrhoids according to modern era. Hence, the diseases of ano-rectal area are the commonest of all the diseases of GI tract.

The incidence of Anorectal disorders are increasing as compare to other disorders, bleeding per rectum is one of the commonest symptom. The lifestyle disorders such as anorectal disorders, hypertension, Diabetes mellitus are also caused due to unhealthy diet, indigestion, change in lifestyle, improper diet schedule and time, improper posture of sitting, mental stress etc. *Sushruta Samhita* has mentioned in *Arshanidanam* that the one who suffers from *Mandagni* which leads to *Vatapra t kopa* alone or in combination with other *Dosas* and causes symptoms such as gudashoola, saraktamalapravrutti, gudadaha etc.

In modern texts, due to improper lifestyle and improper diet there is indigestion of food which cause hard stools. Hard stools are main causative factor for haemorrhoid, which leads to constipation and increased abdominal pressure. This leads to increased venous engorgement of the haemorrhoidal plexus and cause of prolapse of haemorrhoidal tissue. This may result into bleeding per rectum, thrombosis, inflamed haemorrhoids. This study aimed at *Annavahsroto-dushti* and its relation in Pathogenesis of *Arsha Vyadhi* and its preventive management. They were well known for...
time immemorial to the physician and surgeon due to the worldwide distribution and trouble caused by them.

**Aim:** Annavahsroto-dushti and its relation in Pathogenesis of arshaVyadhi.

**Objective:** To Review Annavahsroto-dushti with respect to arsha vyadhi.

**Materials and Methods**

Thorough review of literature related to Srotas and relevant topics was done through the Ayurved Compendia, various other Ayurved texts and textbooks of contemporary science. The references from internet and journals were also critically reviewed. The study had initiated for Affirmation of Annavaha Srotodusti leads toarsha vyadhi.

**Review of Literature**

**ANNAVAVA SROTAS**

Channels carrying anna (food) is called annavaha srotas.

*Annava Srotas* is the channel for transportation, digestion and absorption of food

*Annava Dhamani* plays vital role in the Priṇan Karma of Rasa Dhātu by carrying paramshukma Tejobhuta, properly digested, PanchbhauticAhararasa to whole of body. *Amasaya* is the storage site of the Panchbhautic Anna and along with the *Vamaparshwa*. Amasaya is considered as Moolasthān with storage point of view, *Annava Dhamanīes* as conduction point of view and *Vamapārśwa* as clinical point of view.

**Mula (root)**

*Annava Srotas* originates from amasaya (stomach) and Vama Parsva

*Annava srotas* are two in number and they have their origin in amasaya (Stomach) and annavaha Dhamani (anna carrying dhamanies)

**Nidan (etiological Factors)**

Excess intake of food, eating during improper time, consumption of unwholesome food, due to impairment in agni

**Annava Srotodusti Lakshana**

When annavahasrotas get injured or damaged it causes distention of abdomen, pain, aversion towards food, vomiting, thirst, blindness, death(4).

Lack of interest towards food, anorexia, indigestion, vomiting sensation

**Arsha**

**Nidana (Causative factors)**

**Nidan of SahajaArsha**:

- Mithya aahara and vihara of matruja and piitruj
- Poorvajanmakarma

**According to Sushruta**

Shonita and shukra vitiatan

**Samanya Hetu :**

**Aaharaj Hetu:**

- Virudha bhojana,Pramitbhojana, Asatmya-bhojana
- Guru, Sheeta, madhura, abhishyandi, vidahi aana seavana
- Matsya, Varaha, Mahisha, Aja- Mansa
- Krusha-Prani Mansa, Shushka Mansa
- Nava shuka Dhanya, Ati-snehapan
- Dadhi, Ikshu Ras, Ksheer etc.

**According to Sushruta**

- Virrudhashana, Adhyashana

**Viharaja hetu**

Streeprasanga, utkutasana, prushtayana, vegavidharana

**Vishesha Hetu**

**VatajaArshas**

- Excessive intake of Kashaya, Tikta, Katurasa
- Ruksha, Sheeta and Frequently taking diets in
· Laghuguna Aharadravyas
· Less intake of food
· extremely less quantities
· Intake of Rukshamadya
· More exposure to wind
· Oversexual indulgence

Pittaja Arshas\(^{(7)}\)
· Excessive intake of food having 
Vidahi properties
· Intake of alcohol
· Anger
· Hot place and time
· Exposure to sunlight and fire
· Over exercise
· Excessive intake of pungent, sour, salty Rasas, Kshara and Ushna, Tikshnaguna Aharadravyas

Kaphaja Arshas\(^{(8)}\)
· Excessive intake of sweet, salty and Day sleeping
· Lack of exercise
· sour Rasas and Snigdha, Sheetaguna of Ahaaradravyas
· Mental inactivity
· Cold place and time
· Exposure to eastern wind

**SAMPRAPTI (PATHOPHYSIOLOGY)**

According to Shushruta due to nidan of arsha mainly due to mandagni it cause vitiation of doshas as single or more along with rakta and doshas move downwards through the mahadhamani reach to the guda which affects gudavalitraya and cause arsha

According to Charaka Arsharoga is produced due to vitititian of all doshas which follows bahya and aabhyantararogmarga and affect gudavalitraya

**Samprapti Ghataka**

Dosha - Tridosha
Dushya - Tvak, mamsa, meda, rakta
Srotas - raktavaha and mamsavaha srotas

Srotodusti - Sanga, siragranthi

Udbhavasthana - amaapakvasayotbhava

Vyaktasthana - Gudavalitraya

Rogmarga - bahya and abhyantarara

Agni - Jataragnimandya

**In the aspect of kriyakala**

These are the six stages of manifestation of disease. It has mentioned only by sushruta. The managementof each shat kriyakaal stages is different. The symptoms are aggravated after the sthana sanshaya avastha. Hence if treatment is given before this avastha then it will not occur.
**CHIKITSA VIVECHANA** (TREATMENT)

In contemporary sciences surgical treatment available for haemorrhoids such as higation and haemorrhoidectomy, rubber band ligation, sclerotherapy, but these surgical procedure having adverse effects such as bleeding, infection, incontience, strangutation etc but surgery is not only option for piles it can be cure by preventive and curative measures.

**Management**

**Preventive measures**
- *Nidanparivarjana*
- *Snehana*
- *swedana*
- Sama agni – keep agni in equilibrium condition
- *Pachana*
- *Deepana*

**Curative measures**
- Medical
- Parasurgical-Kshara* sutra,Agni karma,Raktamokshana
- Surgical- Chedana

**Apathya in Arsha**

**Diet:**

Heavy food, Vishtambhi, Vidahidravya like Chilies, Spices, food stuffs made of rice, fried food, Maidaproduet,

excessive intake of oils, Non vegetarian foods Curd, etc.

**Habits:**

Lack of exercise, sleep in day time, Constant sitting on hard objects, Excessive riding, straining during defecation etc.
Pathya(9)

Diet:

Milk, Takra (Mattha), wheat, Cow ghee, Green vegetable etc.

Habits:

Regular diet, exercise, proper sleep, etc.

Observations and Results

Health defines Ayurveda as “Samadosha, samagni, samadhatu malakriyah Prasannatma indriyas manah swath abhidayate (10)” For healthy life agni should be in sama avastha.

In this modern era due to improper diet habits and changing in lifestyle cause annavahasrotadusti and causes agnimandya leads to indigestion which is causative factors of various disorders. Hence irrespective of any disorder agni should be consider first. Hence agnipariksha is so important to examine by physician.

Discussion

Arsha is a problem related to life style, age, occupation and dietary factors. It is a very terrible condition, patient is afraid of defecation because of pain with bleeding per rectum. Hard stools, improper bowel habit are most common symptoms. Due to that it hampers the digestive system and cause agnimandya. Hence agni should be in samaavastha first. Ayurvedic formulations which gives deepanpachana effect can be used and improve the malapraavrutti. Also intake of proper diet is necessary and nidanparivarjana is important factor to be considered.

Conclusion

- Annavaha srotodusti is mainly responsible for indigestion.

- Agnimandya is main reason for Arsha which is due to improper diet habit which leads to annavahasrotodusti.

- Therefore if person follows proper diet it keeps agni balance and proper bowel habits that will prevent from anorectal disorders.

- Hence, person should focus on the diet plan what should eat and what should be avoided.

- Prevention is always better than cure.

Ethical Clearance- Taken from Institutional Ethical committee

Conflict of Interest: NIL

Source of Funding- Self

References

Association of Poor Sleep with Low Back Pain among Symptomatic and Asymptomatic Population: A Research Protocol

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Abstract

Background and Objectives- Low back pain (LBP) is the key contributor to ages of disability. Although Low back pain puts an immense economic strain on healthcare services, this disease is responsible for impacting people’s daily lives. Low back pain is a widely common illness impacting persons of all ages. Sleep is an intricate and fundamental organic element with the social objective of keeping up homeostasis by methods for various physiological frameworks. Sleep quality has gained interest for people with musculoskeletal pain conditions as a contributing factor to the outcome. Sleep consistency has created consideration as a risk factor to the outcomes for people with MSK pain disorders. Some studies show a bidirectional link between intensity of pain and sleep quality. Recent attention to the combination of inflammatory processes in pain on the one side and sleep on the other, contributes to neuro-immunological mechanisms that may contribute to the near connection between pain and sleep.

Aim and Objectives- To study association of poor sleep with low back pain among symptomatic and asymptomatic population.

Materials and Method- All asymptomatic and symptomatic individuals with low back pain in the group between 35-50 years of age will fill the survey using Visual Analogue Scale (VAS) and Insomnia Severity Index Questionnaire, and further data will be analyzed.

Result- The parameters of outcome measures of both the group of low back pain individuals will be analysed using the statistical test namely students paired T-test.

Conclusion- Based on the previous studies we assume that there should be a positive association between both poor sleep and low back pain.

Key words- Low back pain, sleep, Visual Analogue Scale, Insomnia Severity Index.

Introduction

Low back pain is the main contributor to ages of disability affecting as many as 84% of all individuals at some point in their lives.¹Low back pain (LBP) is characterized as pain between the costal margins and lower gluteal folds, which is typically followed with severe movement restriction, may be correlated with
leg-related pain (‘leg pain’) and may not be correlated with fracture, direct trauma or systemic diseases such as neoplastic, infectious, vascular, metabolic or endocrine related processes.\(^2\)

Low back pain is a very common symptom. Low back pain is estimated to be the world’s leading cause of disability and the sixth largest contributor to the global disease burden, suggesting a prevalence of 9.4\(^{\circ}\).\(^3\) It occurs in high-income, middle-income, and low-income countries and all age groups from children to the elderly population. Globally, years lived with disability caused by low back pain increased by 54% between 1990 and 2015, mainly because of population increase and ageing, with the biggest increase seen in low-income and middle-income countries. Low back pain is now the leading cause of disability worldwide.\(^4\)

Low back pain is a global concern which is growing slowly leading to population ageing. Low back pain can be said to affect all age groups and is commonly related to sedentary jobs, smoking and obesity.\(^5\)

Non-specific LBP is characterized as LBP not induced by any familiar cause\(^6,7\) which accounts for 90–95 percent of LBP cases. Non-specific LBP is reported to have an eighteen percent incidence point.\(^6\)

Although Low back pain puts an immense economic strain on healthcare services, this disease is responsible for impacting people’s daily lives. Low back pain is a widely common illness impacting persons of all ages.\(^6,8\)

In 2015 Global Burden of Disease Report, where illnesses are evaluated by how much hindrance they cause in ages lost with inability, Low back pain was positioned as world’s biggest supporter of disability.\(^9,10\)

Sleep plays a vital part in human development, metabolism, and cognitive and physical rehabilitation. Past findings have demonstrated that sleep deficiency and prolonged lack of sleep may have a detrimental effect on executive performance, thinking and memory, response rate, auditory alertness and mood. Insufficient sleep hours have also been shown to influence on metabolism and endocrine activity and awareness.\(^13\)

Sleep disorder is a rising public health concern worldwide. It can be influenced by environmental, cultural and behavioural factors and is estimated to have a prevalence of 10 to 48%. Sleep deficiency and sleep disorders can increase the risk of chronic hypertension, overweight / obesity, cardiovascular disease and psychological issues, thereby impacting both the quality of sleep and the overall quality of life.\(^3\)

Sleep disorder is a rising public health concern worldwide. It can be influenced by environmental, cultural and behavioural factors and is estimated to have a prevalence of 10 to 48%. Sleep deficiency and sleep disorders can increase the risk of chronic hypertension, overweight / obesity, cardiovascular disease and psychological issues, thereby impacting both the quality of sleep and the overall quality of life.\(^3\)

Poor sleep additionally gives a significant and complex threat factor for a wide number of physiological issues and physical disorders, dementia, chronic pain, DM and all-cause mortality.\(^15,16,17\) Poor sleep quality among people with chronic low back pain appears to be related to worse pain, affects poor physical function, and pain catastrophizing.\(^18\)

Sleep is important and its deprivation will result in serious consequences. Experimental studies in healthy volunteers (without pain) have shown that induced sleep deprivation contributes to musculoskeletal pain and...
enhanced pain sensitivity to noxious stimuli through either a decrease in sleep length or a disturbance in sleep architecture.11

Back pain and poor sleep quality are public health issues. Sleep deprivation has created consideration as a risk factor to the outcomes for people with MSK pain disorders. Some studies show a bidirectional connection between pain and sleep quality.1,19 Recent attention to the association between inflammatory processes in pain, on one side, and sleep, on the other, leads to neuro-immunological mechanisms which may lead to the close correlation between pain and sleep.19

Sleep recovery in healthy volunteers after a duration of prolonged sleep deprivation has also been shown to produce an analgesic effect close to that caused by non-steroidal anti-inflammatory drugs. In addition, improved sleep quality in patients with debilitating conditions, such as osteoarthritis and chronic musculoskeletal pain, is substantially associated with pain intensity reductions. Eventually, earlier work also shows a clear correlation between sleep disturbance and musculoskeletal pain production. A study in Finland found that sleep disruption is a good predictor of adolescents experiencing low back pain.11

Murase Kimihiko et. al. (2015) performed a study showing that an enormous example of everyone not just indicated a peak going rate of knee and LBP, yet in addition that LBP and knee were firmly connected to less sleep and poor sleep standard, independent of other elements.15

Min Chun Young et. al. (2018) performed a study that found the relation between self-respond sleeping period and musculoskeletal discomfort in the older Korean community by making use of the KNHANES dataset. This research, stated that excessive sleep period is prevalent in any musculoskeletal pain individuals and is more common in multiple site joint pain subjects.20

Vinstrup et Jonas. Al. (2019) records strong dose-response relationship between arbitrary baseline sleep ratings and low back pain follow-up risk for health care staff, with substantial correlations observed in both sensitivity tests. Taking into consideration the high incidence of musculoskeletal conditions in occupational health groups.14

Research investigating the effect of sleep disruption on low back pain severity have shown a substantial correlation between sleep quality and intensity of pain, exhaustion, subsequent-day function and psychological distress. Individuals with low back pain who have sleep issues and more extreme pain have also been reported to be at higher risk of being hospitalized for low back pain than people with good sleep quality. These findings suggest that poor quality of sleep may be associated with exacerbations of low back pain; however, no direct association has been measured in low back pain to date.11

A critical issue in standard clinical practice is that sleep disruptions make treatment of LBP more difficult, suggesting a greater risk for it to become chronic. Longitudinal routine practice studies would be useful in exploring this and in developing a potential research agenda in this area; If sleep disturbances were associated with a higher risk of LBP becoming chronic, it would make sense to routinely assess and potentially treat the quality of sleep in LBP patients (whether or not they are seeking care), and to develop randomized controlled trials to determine whether treating sleep disturbances improves the outcome of LBP treatment.21

Although it has been shown that poor sleep and LBP are associated, but a little scientific information is available between the association of poor sleep in asymptomatic individuals and symptomatic low back pain individuals and vice versa.

Objectives
- To evaluate effect of poor sleep on LBP
- To evaluate poor sleep association with low back pain in asymptomatic individuals

Methods
This study will be carried out at Ravi Nair Physiotherapy College, Musculoskeletal OPD, Sawangi (Meghe), Wardha, Maharashtra, India after approval from Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences, Deemed to be University.

Study design: Observational study

Study setting: Ravi Nair Physiotherapy College, Musculoskeletal OPD, Sawangi (Meghe), Wardha, Maharashtra, India
Participant: All asymptomatic and symptomatic individuals with low back pain in the group between 35-50 years of age

Inclusion criteria
- Both male and females
- Age group 35-50 years
- Population with low back pain
- Asymptomatic population
- Population having baseline LBP

Exclusion criteria
- Low back pain with radiculopathy
- Chronic low back pain patients
- Patient on sedatives

Variables

Outcome measures:
- Insomnia Severity Index
- Visual Analogue Scale

Data source measurement
- **Insomnia Severity Index** – The (ISI) contains seven items that measure insomnia’s severity and consequences, and all items are graded using a five-point Likert scale (0 = no problem; 4 = very severe problem). A total score is obtained after summing up all the responses and the total score ranges from 0 to 28, where 0–7 indicates insomnia, 8–14 indicates insomnia at the subthreshold, 15–21 indicates moderate insomnia, and 22–28 indicates severe insomnia. Reliability for Insomnia Severity Index (intra-class correlation coefficient, ICC2,1−0.84) (Pearson’s coefficient r−0.45). 22

- **Visual Analogue Scale** – The Visual Analog Scale (VAS) is a straight line of 10 cm with endpoints describing extreme limits such as ‘no pain at all’ and ‘pain as severe as it may be.’ On the line between the two endpoints the patient is asked to mark his pain level. The distance between ‘no pain at all’ and the mark determines then the pain of the subject. It is a valid and reliable tool for rating low back pain. \( r = 0.767 \) to \( r = 0.943, p = 0.000 \). 25

Study size – 200

Group A – 100 Symptomatic low back pain individuals

Group B – 100 Asymptomatic individuals

Sampling technique is simple random method.

Procedure

Institutional ethical clearance will be obtained before beginning the study. 200 subjects, between the age group of 35-50 years with low back pain symptomatic and aged matched asymptomatic subjects from Ravi Nair Physiotherapy College and Musculoskeletal OPD will be explained about the study procedure based on the inclusion and exclusion criteria. A proper consent will be taken from the patients and the patients will be explained about the study. The patients who wish to participate in the study will be taken into isolation in order to respect their privacy. After the consent is taken, with sample size of 200 two groups will be divided i.e. Group A- 100 symptomatic low back pain individuals and Group B- 100 asymptomatic individuals. VAS ratings will be taken for Group A subjects having low back pain and Insomnia Severity Index scale will be evaluated for both the groups of symptomatic and asymptomatic individuals. Subjects will be asked to fill the questions with the answers best suited to them. Data will be collected and documented for data analysis.

Expected Result

The result would include if poor sleep is a risk factor for low back pain or vice versa i.e if both are associated with each other. Using the INSOMNIA SEVERITY INDEX (ISI) And VISUAL ANALOGUE SCALE (VAS) the study would be carried out. Once the study result is complete, statistical analysis will be analysed using paired T-test and presented in the form of research paper.

Discussion

The point of this study is to check the relationship of poor sleep with LBP among both symptomatic and asymptomatic people. In spite of the fact that in
numerous investigations some relationship between poor sleep and low LBP have been appeared, however an exceptionally low logical data is accessible which shows the relationship of poor sleep with asymptomatic people and symptomatic people and the other way around.

To draw a close attention, this research will look at the association between the two that is poor sleep and LBP and how they are associated which can be very helpful in treating the LBP cases. Even, to make sure that these factors like poor sleep if hampering the treatment for LBP. To check the relation between the both and to check if poor sleep leads to LBP or if LBP leads to poor sleep or if both are interlinked to each other using the Visual Analogue Scale and the Insomnia Severity Index.

Conflict of Interest- None

Funding support- None

References


Effectiveness of Pippalyadi and Suranadi ointment in management of Arsha (Haemorrhoids)

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Abstract

Arsha is a common anorectal disease so associated with bleeding per anum. It can be compared to Haemorrhoids according to modern science. Haemorrhoids are dilated veins in the anal canal. Through all the modern modalities of treatment in haemorrhoids are universally accepted but all have some or other limitations. In Ayurveda various measures have been mentioned for the management of Abhyantar Arsha, among which lepa karma is one of them. Various study have been done on the application of various lepa so prepared have been conducted depending upon the sign and symptoms. In this study the effectiveness of Pippalyadi and Suranadi ointment were compared which were so mentioned in bhaishajya ratnakalari. In this present research work efforts were made to provide the treatment which is non-invasive, easy to implement, effective and not need any hospitalization. In this study 30 patients of Arsha were selected as per criteria of selection randomly irrespective of religion, socio-economic status and were divided in 2 groups of 15 patients each. Group-A was treated with Pipplyadi ointment advised for 15 days twice a day as local application. Group-B allotted Suranadi Ointment given twice a day for local application for 15 days and accordingly the result were assessed. Pippalyadi and Suranadi ointment were prepared according to the texts and the ointment was used for local application on the patients of Arsha. Lastly, it was concluded that both the ointments have shown significant results in the patients without any side effects.

Keywords: Arsha, Haemorrhoids, Pippalyadi and Suranadi ointment, Lepa, Raktaastarava, Degree of prolapse.

Introduction

Ano-rectal disorders are progressively increasing in the society. Out of many of the causes, some important are sedentary lifestyle, irregular and inappropriate diet, prolonged sitting or standing and certain psychological disturbances too. Arsha is one among the ano rectal diseases which occurs in Gudapradesha, which is a sadhyopranahara Marma[1]. In Arsha bleeding per anum is the principal symptoms. Arsha is a gift of modern diets and busy lifestyles and many people are suffering from some sort of Anorectal disorder, it may be simple constipation to complex carcinoma, in which prominent disorder is Arsha. It is manifested due to multifold factors viz. disturbed lifestyle or daily routines, improper or irregular diet intake, prolonged standing or sitting, faulty habits of defecation etc. which results in derangement of Jatharagni leading to vitiation of Tridosha, mainly Vata Dosha. These vitiated Doshas get localized in Guda Vali and Pradhana Dhamani which further vitiates Twak, Mansa, and Meda Dhatus due to Annava shrotodushhi leads to development of Arsha.

In modern medical science Arsha can be compared with haemorrhoids. Hemorrhoid often described as “varicose veins of the anus and rectum”. Hemorrhoid are dilated, tortuous or varicose veins occurring in relation to the anus and originating in the epithelial
plexus formed by radicals of the superior, middle and inferior rectal veins. While other scientists considered it as displacement of anal cushions. Thomson wrote that it occurs through vascular hyperplasia in the anal sub mucosa, possibly through dysfunction of the shunts. According to the John Goligher pile’s seems to be more appropriate for this condition as all hemorrhoid do not bleed.

Haemorrhoids are divided into two categories- internal and external haemorrhoids. Internal haemorrhoids means it is within the anal canal and internal to the anal orifice and the external haemorrhoid is situated outside the anal orifice and is covered by skin. The two varieties may coexist and the condition is called intero-external haemorrhoids[3].

In the management of Haemorrhoids, the procedures, which are in practice at present in modern surgery, are laser surgery, rubber band ligation, sclerotherapy, bipolar diathermy, infrared photoocoagulation, cryosurgery, infra-red coagulation and hemorrhoidectomy, but all procedures have their limitations[4]. Though all the modern modalities of treatment in haemorrhoids are universally acceptable but all have some limitations. In ancient Ayurvedic compendia various palliative measures have been mentioned in the management of Abhyantar Arsha, lepa karma is one among them. Lepa is one of the type of abhyantar sneha. Basically lepa is application of paste of medicated plants mixing with oil. According to sushruta thickness of the lepa should be around 3-5 mm, whereas according to Acharya charak it is 1/3rd of Angustha. Lepa is prepared by grinding and crushing the herb together into a fine paste form. If the drugs are in dried form then it is to be made into paste by triturating it into paste with water, milk, ghee or oil.

In this present research work efforts were made to provide the treatment which is non- invasive, easy to implement, effective and were not need any hospitalization. To fulfill the above criteria the present research study were conducted to compare the effects of Pippalyadi lepa and Suranadi lepa in management of abhyantar Arsha, which are described in Bhaishajya Ratnavali 9th Chapter[6]. Application of lepa over internal haemorrhoids and its preparation were a difficult job therefore in order to make that easy, ointments were prepared out of the same ingredients.

**Aim and Objective**

- To evaluate the efficacy of Pippalyadi Ointment in management of Arsha.
- To evaluate the efficacy of Suranadi Ointment in management of Arsha.
- To compare the effects of Pippalyadi Ointment and Suranadi Ointment in management of Arsha

**Materials and Methods**

**Drug Review**

Pippalyadi ointment and surandai ointment were prepared according to classical reviews so mentioned. The preparation has been described in detail and pharmaceuticals analysis is given below.

**Pharmaceutical analysis:**

The raw materials were collected from reliable source and were authenticated from Department of Dravyaguna and it were analysed in Pharmaceutical Laboratory, Sawangi Wardha and the findings are as follows:

1) **Pippalyadi ointment**

- Loss on drying at 105°C : 1.053
- Spreadability: 4.7gm.cm/sec
- pH : 5.7
- Peroxide value: 5.82
- Iodine value: 17.28

2) **Suranadi ointment**
§ Loss on drying at 105°C : 1.21
§ Spreadability : 6.5 gm.cm/sec
§ pH : 6.12
§ Peroxide value : 6.28
§ Iodine value : 15.37

Organoleptic characters

1) **Pippalyadi Ointment**
- Color : Greenish
- Texture : Smooth
- Odour : Characteristic

2) **Suranadi ointment**
- Colour : Yellowish
- Texture : Smooth
- Taste : Characteristic

**Clinical Study**

In this study total 30 patients of Arsha were selected and divided in two groups and Pippalyadi and Suranadi ointment were administered. Group A was treated with Pippalyadi ointment for 14 days twice daily as local application whereas Group B were given Suranadi ointment for 14 days as local application.

**Inclusion criteria**

§ Diagnosed without any major systemic disorder’s cases of first and second degree internal haemorrhoids.

§ Age group of 20 to 50 years, irrespective of their sex, occupation, & economic.

**Exclusion criteria**

§ Haemorrhoids that are thrombosed, third degree and fourth degree.

§ Haemorrhoids associated with fissure in ano, fistula in ano, perianal abscess, rectal polyps, rectal prolapse and rectal CA.

§ Patients having tuberculosis, AIDS & Hepatitis, Ulcerative colitis, Crohn’s disease & Pregnant women.

**Diagnostic criteria**

Diagnosis was made on the basis of physical examinations by performing thorough P/R examination i.e inspection, palpation, digital and proctoscopic examination.

**Investigations**

Routine hemogram, blood sugar, routine and microscopic examination of urine and stools were carried out.

**Grouping and posology**

In this present study total 30 patients were take which were further divided into two groups,

GroupA and GroupB each containing 15 patients by using simple randomisation method. In this study 30 patients of Arsha were selected as per criteria of selection randomly irrespective of religion, socio-economic status. All these patients were diagnosed with the help of criteria of diagnosis. Patients attending O.P.D of the hospital were examined prior to the start of treatment with respect to the Performa. The treatment protocol comprised of. Application of paste. In both the groups. In GroupA Pippalyadi lepa Was applied whereas in Group B. Suranadi lepa was applied.

The total duration of the treatment was 4 weeks with regular follow up at 7th day, 15th day and 30th day. All the patients were advised to take light and easily digestible diet and avoid incompatible foods.

**Observations were recorded and noted as follows:**

| 1) Total number of patients recruited in the Study | 35          |
| 2) Number of patients who completed study       | 30          |
| 3) Number of patients who had taken Pippalyadi Lepa | 15          |
| 4) Number of patients who had taken Suranadi Lepa | 15          |

Preparation of ointments Preparation of **Pippalyadi** ointment:
Grade 1  | No prolapsed. Just prominent blood vessels.
Grade 2  | Prolapsed upon bearing down but spontaneously reduce.
Grade 3  | Prolapsed upon bearing down and require manual reduction.
Grade 4  | Prolapsed and cannot be manually reduced.

All the drugs viz. Pippali, Saindhav, lavan, Kushta, Shirish and Snuhi are made into a fine powder. Til tail is taken into clean stainless vessel and placed over mild heat until it starts foam appearing. Then beewax is added to 1/5th of til tail, when all the wax is completely melting in oil, it is filtered and kept in another vessel. It was used as base for preparation of ointment called as siktha tail. In this tail fine powder of all above drugs were added and stirred. This mixture then attains thicker consistency as wax cool down and made into a soft paste.

**Preparation of Suranadi ointment:**

All the drugs viz. Suran, Haridra, Chitrakmool, Sudhha Tankan and Guda are made into a fine powder. Til tail is taken into clean stainless vessel and placed over mild heat until it starts foam appearing. Then beewax is added to 1/5th of til tail, when all the wax is completely melting in oil, it is filtered and kept in another vessel. It was used as base for preparation of ointment called as siktha tail. In this Siktha tail fine powder of all above drugs along with Guda were added and stirred. This mixture then attains thicker consistency as wax cool down and made into a soft paste.

**Mode of action**

**Mode of action of pippalyadi ointment:**

**Assessment criteria**

**Subjective Criteria** - presence of clinical signs and symptoms of abhyantar Arsha.

1. Constipation

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Grade</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Present</td>
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</tbody>
</table>

2. Bleeding per rectum

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Grade</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Present</td>
</tr>
</tbody>
</table>

**Objective criteria**

**Statistical Analysis**

Subjective symptoms were statistically analyzed by applying nonparametric test like ‘Wilcoxon Sign Rank test’ within group to compare the results before and after treatment results. For the comparison between the group for subjective criteria ‘Mann Whitney U’ test was applied. Level of significance were taken at 5%.

**Assessment of Results**

Assessment of the result was done based on the relief of signs and symptoms i.e bleeding per rectum , pain, constipation, degree of prolapse, discharge and reduction of pile mass.

**Observation and Result**

In was so observed that out of the total 30 patients which were further divided in two groups of 15 each. The first group i.e Group A in which pippalyadi ointment was used showed a significant difference in the condition when the follow up was taken on Day 7, Day 14 and Day 30. The ointment showed gradual improvement in the symptoms including bleeding (raktastrava) and the degree of prolapse on the Day 30 as compared to that on Day

0. Whereas when comparison was done within each follow up in the case of constipation (malavastambha) no significant improvement was observed in Group A.

Looking over the second group i.e Group B in which suranadi ointment was used it showed the same results,
stating much improvement in the symptom of bleeding. Further showing a bit less improvement in the degree of prolapse as compared to the Group A in the followups. And in the case of constipation this ointment showed very slow response and stating lesser improvement in the Day 14 and Day 30 followups. This could be better understood by the table so given below

**Pippalyadi ointment -Group A**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>7th day</th>
<th>14th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding (Raktastrava)</td>
<td>18.2%</td>
<td>45.5%</td>
<td>90.9%</td>
</tr>
<tr>
<td>Constipation(Malavastam bha)</td>
<td>36.4%</td>
<td>27.3%</td>
<td>45.5%</td>
</tr>
<tr>
<td>Degree of prolapse</td>
<td>28.9%</td>
<td>55.3%</td>
<td>57.9%</td>
</tr>
</tbody>
</table>

**Suranadi Ointment -Group B**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>7th day</th>
<th>14th day</th>
<th>30th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding (Raktastrava)</td>
<td>0%</td>
<td>54.5%</td>
<td>90.9%</td>
</tr>
<tr>
<td>Constipation(Malavastam bha)</td>
<td>20%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Degree of prolapse</td>
<td>26.5%</td>
<td>38.2%</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Discussion**

The number of patients so found were more among the lower economic group. It was also found that they had irregular dietary habits and were consuming spicy food on regular basis. It was also observed that Out of 30 Patients in the study, 05[16.7%] patients were doing moderate nature of work, 13[43.3%] patients were having sedentary work, while 12[40%] patients were having strenuous work in nature.

Most common symptoms of Arsha includes Agnimandya, Vankshana Shool, Svara Krishata, Asarata,Klama, Jwara, Shotha,Vaman, Peenasas, Arochak, Angamarda and many more[7]. The basic line of treatment for Arsha includes Ksharkarma, Bhes'hajhikitsa, Agnikarma and Shastrakarma[8]

Applicaton of various ointment including Kasis, hartaala, saindhav, karvir, vidang, karanj, krutvedhan, jambuk, arka, bhumi, amalaki, danti, chitrak, alark, snuhi sidd tail are used[9]. Vegadharana, atistreesanga, utkatukasana, prushtayana, atapasevana, atijalapana, vanama, basti, poorva desha vayu sevana, viruddha dravya in rasa, veerya, vipaka these things should also be avoided to maintain good health and prevent further complications.

Pippalyadi Ointment contains Pippali having Laghu, Snigdha, Tikshna guna and madhura, katu rasa which is acts as Vatakaphashamaka. It is also having properties like Antibacterial, Antifungal, Anthelmic. Saindhav lavan, Kushta is having properties like Kaphavatajita, Raktashodhaka. Shirish acts as Shothahara, Tridoshahara. Shirish and Snuhi are having properties like kashay rasa and astringent by nature immediately helps in stopping bleeding due to its Rakta Stambhana Karma. So due to combine effects of all drug bleeding,
inflammation, pain is stopped due to this Ointment which was act locally to relieve the pain and bleeding in arsha.

**Suranadi Ointment** having Suran, Haridra, Chitrakmoool, Sudhha Tankan and Guda. Surana is having properties like Raktapittakara, Deepana, Kaphahara, Ruchya having katu and kashay ras which stop bleeding in hemorrhoid. Haridra is

Kushtaghna, Lekhaniya, Kandughna, Vishaghna. Chitrak is Shothahara, Kaphavatahara, Arshohara, Shulahara,; locally act as shoolhara and relive pain and stop the bleeding.

Statistical analysis shows that both the ointments i.e Pippalyadi and Suranadi were found to be effective in treating the Degree of prolapse, Raktastrava and all the other symptoms of Arsha.

**Conclusion**

The prevalence of Haemorrhoids are progressively increasing in the society due to various factors like disturbed lifestyle, improper or irregular diet intake, prolonged standing or sitting, faulty habits of defecation.

Modern treatment in hemorrhoids are universally acceptable but all have some limitations and chances of recurrence of hemorrhoid are often more. In ancient Ayurvedic texts comforting measures have been mentioned in the management of Abhyantar Arsha, Lepa karma is one among them. It can be concluded that Arsha could be healed by the application of Pippalyadi and Suranadi Ointment. Effect of Intervention of Pipalyadi ointment seen significant on Day 30 as compare to Day 0 for Bleeding (Raktasrava) and Degree of prolapsed. It was observed that there is no significant difference statistically found in constipation among the follow up i.e. Day 7, 14 and Day 30. Similarly, Suranadi Ointment has also found effective in Bleeding (Raktasrava) and Degree of prolapsed and not observed significant in constipation. It was found to be effective in treating various symptoms and the notable point was that there were no adverse effects of the drugs after treatment.

Yet there are few limitations of the study. This study can be done more sample so that the results can be generalized. Along with this intervention of local application, few additional oral drugs can be added to obtain better results, as arsha has also treated with deepan, pachan drug to improve agni of patients and other modern modalities could also be kept in mind.

**I.E.C- Permission is taken from committee ref no. is DMIMS(DU)/IEC/2017-18/7252**

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Management of Phenylketonuria: Current and Future Perspectives

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Abstract

Phenylketonuria (PKU) is an inborn error of phenylalanine (phe) and tyrosine (tyr) metabolism. It is an autosomal recessive disease occurred due to deficiency of liver enzyme phenylalanine hydroxylase (PAH). Hence, phe is not converted to tyr and phe is accumulated in the body. Phe thus channeled to alternative routes of metabolism and forms Phenylketones excreted in urine. Early treatment is essential to prevent mental retardation and other intellectual disabilities. Dietary treatment remains the main cornerstone to manage PKU since last 3-4 decades. A diet low in Phe supplemented with special amino acids formulas must be started soon after diagnosis within seven days of life. Inspite of good results obtained from dietary treatment in PKU, still there are some issues with palatability of the dietary formulations. There are also issues of nutritional deficiencies of vitamins like calcitriol and cobalamin (B12). Poor cognitive and executive functions have been observed in patients who do not follow proper dietary treatment. Attempts have also been made to increase the palatability of food under dietary management. Role of large neutral amino acids (LNAAs) and glycomacropeptides (GMP; found in bovine milk) as a newer dietary management have also been explored. In recent era, advances occurred in terms of genetic therapy and enzyme replacement therapy which opened a new door towards management of PKU. In this review, various treatment aspects of PKU are discussed and explored.

Keywords: Phenylketonuria, phenylalanine hydroxylase, phenylketones

Introduction¹-⁶

Phenylketonuria (PKU) is an inborn error of phe and tyr metabolism. It is caused mainly due to mutations in the gene coded for phenylalanine hydroxylase (PAH) which is located on long arm of chromosome 12. This mutations lead to decrease catalytic activity of PAH which affects the catabolism of phe (Figure 1). PAH is located in liver and requires tetrahydrobiopterin (BH4) as a co-factor to convert Phe to tyrosine (Tyr) (Figure 1). In PKU, accumulation of phe is due to deficiency of either PAH or its cofactor BH4. Accumulated Phe take alternative routes to form phenylketones like phenylpyruvate, phenyllactate, phenylacetate which are excreted in urine. These ketone metabolites lead to severe central nervous system disability which if left untreated cause irreversible damage. Other clinical features associated with untreated PKU may include sensory and motor dysfunction, skin manifestations and seizures. Changes in behavior and psychic disturbances can become apparent as the age progresses.

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Early diagnosis is essential in PKU to prevent irreversible damage to brain. Currently newborn screening (NBS) program is undertaken for initial diagnosis in the first week of life and further screening can be done for BH4 responsiveness in case of atypical PKU. The incidence of PKU varies widely around the world. The average incidence is 1:10000 live births worldwide. India has lower incidence (1:25000 live births) as compared to western countries (1:1500 live births). At present, there is no cure for PKU. However, the cornerstone of the management of PKU is dietary therapy. Diet low in Phe supplemented by amino acids formulations is recommended. Early diagnosis by NBS along with commencement of dietary therapy has played a role in prevention of intellectual impairment but still neurophysiological and neuropsychological impairments persist in already treated patients of PKU. Moreover, some issues are also there with vitamin deficiencies like calcitriol and B12. Nowadays attempts have been made to cope up with issues occurring with dietary therapy. Advanced food and formulas which increase the compliance towards dietary therapy for example incorporation of large neutral amino acids (LNAAs) and glycomacropeptides (GMP) have been studied in detail and introduced. Still further studies are also required in this direction to establish their full impact. Cofactor BH4 responsive patients respond well to BH4 therapy with success rate of only 30%. Recently, newer modalities came into existence and under research like enzyme replacement/ substitution therapy and gene therapy. In this review, we aim to summarize current and future perspectives of management of PKU.

**Current Treatment**

**Diet therapy**

Phe is an essential amino acid. Apart from protein synthesis, phe helps in synthesis of tyr and in turn helps in synthesis of important metabolic substances derived from tyr. In PKU, defect in the conversion of Phe to Tyr makes Tyr an essential amino acid. Therefore, the technical challenge of the dietary treatment is to
formulate a phenylalanine-controlled diet that allows the reduction of systemic Phe concentration, satisfactory tyr provision, and optimal growth and development. Diet low in Phe remains the mainstay of the treatment in PKU. This should be supplemented with special amino acids formula so that proper growth can be ensured. During infancy, Phe free milk lowers the blood levels of Phe. As levels approach the therapeutic range, phenylalanine is added using measured amounts of normal milk and then adjusted until serial blood controls have stabilized. When child puts on solid diet, the diet is progressively adapted with the following main principles: High-protein foods (meat, fish, eggs, dairy, and wheat products) are excluded. Foods with low protein content (milk, vegetables, and fruits) are used to meet the required amount of Phe. Diet restriction is somewhat easier in infancy as it is mainly controlled by parents but it is difficult as the child gets older due to his/her likes and dislikes with the food items. Other important factor that affects the dietary management is the blood Phe concentrations which varies from country to country. Consequently, compliance with the diet is often poor, especially when the patient reaches adolescence, as evidenced by poor control of blood Phe concentrations in this age group. Long-term maintenance of the diet is important for proper management, because it has been observed that patients find it difficult to return to the normal dietary regimen after a long period of unrestricted dietary intake. 7-10

Earlier it was believed that continuation of dietary therapy after childhood has no role because excess phe has no any role after brain development. But some studies showed poor scholastic performance after discontinuation of diet therapy. Moreover, females having PKU when reached to their reproductive age have higher risk of having baby with maternal PKU syndrome. This can be manifested as small brain size and poor brain development, growth retardation in-utero, cardiac defects and a characteristic facial appearance in the affected infant. As a result of these studies, it was recommended that diet therapy should be continuing life-long. 11, 12

Although Phe restricted diet is successful in curtailing intellectual impairment in PKU patients, there are certain issues (non-compliance) still remain with current diet therapy. These are poor compliance to synthetic diet formula, persistence of neurological or psychosocial issues and poor quality of life inspite of early intervention, deficient nutritive supply, financial burden of special diet, difficult dietary regimen to follow, psychosocial and emotional factors, knowledge of disease and its outcomes, attitudes towards healthcare providers, no reimbursement of food supplements in some health care systems. There is an existence of problem in the diet therapy in PKU patient when provided with aspartame (L-aspartyl-L-Phe methyl ester). It is commonly used as an artificial sweetener and it releases Phe, L-aspartic acid and methanol when gets metabolized. Hence, aspartame should be avoided. Stringent diet therapy leads to nutritional deficiencies. The reported deficiencies are vitamin D, B12, calcium and iron which further aggravate the symptoms in PKU patients. 3, 8, 13-16 These issues stimulated mankind to have other alternatives to be made in diet therapy.

**Tetrahydrobiopterin (BH4, Sapropterin) treatment**

BH4 is a natural cofactor required by PAH for the conversion of Phe to Tyr. Some mutations are also associated with BH4-sensitive phenotype of PKU. The benefit of administering synthetic preparation of BH4 in patients with non-classical (atypical) PKU was first studied by Schaub et al. Pharmacological doses of BH4 may reduce the blood Phe levels was first demonstrated by Kure et al. Thus the patients were called BH4-responsive. Two formulations of BH4 have been studied clinically (6R-BH4 dihydrochloride and sapropterin dihydrochloride) but only sapropterin dihydrochloride is approved by the US Food and Drug Administration, the European Medicines Agency, and in Japan for therapeutic use. A single daily dose of 10-20 mg/kg is sufficient to maintain stable blood Phe level over 24 hours. There are other benefits of BH4 treatment which were also observed like improvement in Phe tolerance, improvement in long-term neurological outcomes, decreased in depression and panic attacks especially in females. It was also observed that BH4 supplementation can be appropriate for patients with milder biochemical phenotype. It is less useful in classical PKU. 3, 17-20

**Newer dietary approaches**

**Large neutral amino acids (LNAAAs)**

Phe, Tyr, tryptophan, and the branched-chain amino...
acids (BCAAs) are considered as LNAAs. They share the common amino acid transporter to cross blood brain barrier. In PKU, high Phe levels compete with entry of other LNAAs which can lead to deficient formation of important neurotransmitters and hence neurological symptoms develops. Keeping this fundamental aspect into consideration, supplementation with LNAAs other than Phe could provide another potential treatment approach. LNNAs exert its effect by competing with phe for transportation at two different sites that is across the blood-brain barrier and across the intestinal mucosa. A double-blind, placebo-controlled study also showed a reduction in blood phenylalanine from baseline of 39% during short-term treatment with LNAAs.13, 21-23

**Glycomacropeptides (GMP)**

During making of cheese, GMP produced as a by-product. It is a 64 amino peptide with no Phe residues and rich in valine, Isoleucine and threonine. Therefore, food made from GMP can be a rich source of protein for PKU patients. Study showed that GMP diet significantly decreases urea production, helped in maintenance of protein concentration and phe utilization. It has also been found that taking diet made from GMP for breakfast promoted satiety and improves compliance.5, 24-26

**Future (Newer) Treatment**

**Gene therapy**

PAH is only expressed in liver. So gene therapy involves production of recombinant PAH gene which is targeted to hepatocytes. Viral vectors like adenoviral and adeno-associated virus (AVV) vectors have been used in various studies to know their role for the transfer of this gene in PKU mouse model. Recombinant AAV vectors have been used to deliver PAH gene to the liver in a mouse PKU model, allowing correction of PAH up to one year. But over the time, the vector lost due to continual turnover of hepatocytes and in turn developed immunity. Yagi et al used an AAV8-pseudotype vector with a self-complementary AAV genome and they had achieved excellent liver transduction and expression of PAH with complete reliving of symptoms and normal blood phe for over one year. Skeletal muscle is preferred over liver for gene therapy due to easy accessibility and longer cell life. However, new strategies are still required to extend gene transfer efficacy.3, 13, 27-29

**Enzyme replacement/substitution therapy**

**PAH-based fusion proteins (Enzyme replacement)**

PAH-based fusion proteins which specifically target PAH to the liver have been explored. Mice with PKU were injected PAH-based fusion proteins intravenously. Decreased in Phe level within several hours has been noted. But this intervention may be less tolerable clinically due to frequent injections of fusion proteins. Moreover model used in this experiment was not homologous to human PKU but still it can be the alternative if homologous model is used.3, 13, 30

**Phenylalanine ammonia-lyase (PAL, Enzyme substitution)**

PAL is used as enzyme substitution therapy. PAL an enzyme normally found in plants and fungi. It catalyzes the removal of amino group of phenylalanine to ammonia and transcinnamic acid, the latter of which is then quickly converted into hippuric acid and excreted in urine (Figure-1 red box). Study done in mouse model by injecting PAL showed decreased Phe level but due to repeated injections immune response was developed which decreases its half-life. To overcome the issue, recombinant PAL (with polyethylene glycol polymers so-called PEGylation) is used to avoid the immune-mediated degradation of the enzyme and thus longer half-life is achieved. Oral route is preferred than the Parenteral route to achieve compliance to therapy. But again, to maintain enzyme stability is a big issue which can be overcome by using encapsulation or using live microorganism as a delivery systems. Use of probiotics like lactobacillus and genetically modified probiotics in transporting and delivering PAL have also been gained attention and tried.3, 8, 13, 31-34

**Conclusion**

Prompt initiation of low-Phe diet immediately after diagnosis made by newborn screening maximally prevents intellectual impairment in early treated PKU patients. Compliance to the diet therapy is the main issue especially in adolescence and adulthood which in turn lead to impaired blood Phe control and poor outcome with respect to psychosocial and cognitive assessments. BH4 supplementation can be appropriate for patients with milder biochemical phenotype (Non-
classical PKU) as compared to Classical PKU. Newer dietary approaches have been introduced to increase compliance to diet therapy. Data suggested that use of LNAAs is recommended only in adult patients who have non-compliance to stringent diet regimen. Addition of GMP into low-Phe diet has shown to improved palatability and some beneficial effects but still further studies should be undertaken to establish its safety and efficacy. Newer and future treatment modalities for PKU are now under development. They are mainly focused on the basic defect of PKU i.e. catalytic activity of PAH. Gene therapy by using suitable vectors to transfer PAH gene to liver and skeletal muscle have been tried. Under enzyme replacement and substitution therapy role of PAH associated fusion proteins and PAL (PEGylated form) have been explored respectively. The latter has reached phase III clinical trials but still its efficiency is the major issue. Studies related to the use of probiotics to produce and deliver PEGylated form of PAL are still ongoing to establish its safety and efficacy. Nevertheless, enzyme replacement/substitution therapy has been found efficacious in mouse model of PKU but still they all require in depth research and clinical testing. These treatment modalities can also be applicable to other inborn error of metabolism and data from this study highlighted the need for development of alternative therapies for PKU patients irrespective of genotype, phenotype or gender.

Source of Funding: Self

Conflict of Interest: None

Search Strategy

We searched Google and PubMed with the terms: “phenylketonuria”, “hyperphenylalaninemia”, and “PKU” in combination with “diagnosis”, “treatment”, “diet”, “tetrahydrobiopterin”, “sapropterin”, “pharmacotherapy”, “gene therapy”, “enzyme replacement therapy”, “large neutral amino acids”, “glycomacropeptide”, “nutritional status”, “management”, “adult”, and “maternal”. We gave preference to papers published within the past 10 years, but did not exclude some important less recent publications.

Ethical Approval: This study did not warrant institutional review board review as no human subjects were involved.

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Correlation Between Knowledge, Attitudes, and Behavior with Blood Cadmium Levels in the Population of Industrial Area

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Abstract

Objectives: The aim of this analysis is to investigate the correlation between knowledge, attitude, and behavior with cadmium level in the industrial population. Methods: This research is a non-experimental study with cross-sectional method. The subjects are 108 respondents live in industrial area. Questionnaires were used for measuring the level of knowledge, attitude, and behavior. Cadmium levels were measured using Atomic Absorption Spectrophotometry (AAS). Results: The result showed a strong correlation between levels of knowledge factor and cadmium with \( r = -0.272^* \) in the population of industrial areas. Meanwhile, knowledge have significant correlation with attitude \( (r = 0.528^{**}) \). Conclusion: Knowledge factor has the significant relationship with cadmium levels in the population of industrial area. The government is suggested to improve the knowledge of the people in the industrial area in order to prevent high levels of cadmium. Therefore, the negative impact of metal waste exposure on health can be minimized.

Keywords: Cadmium, Knowledge, Behavior, Attitude, Industrial area

Introduction

Recently, industrial sector in Indonesia has been significantly increased by the time. From the data obtained from the Statistics Indonesia database in 2017, various kinds of industries about 33.577 have already growing rapidly and predicted to continue to expand (1). This is the case with data provided by CEIC (World of Macroeconomic Data) which reports that the Indonesian industry prediction index increased by 2% in February 2020, which shows an increase of 0.8% from February 2020 (2).

With the increase in the number of industries, it will certainly be very beneficial for the country, especially in terms of improving social and economic welfare. If viewed from the other side -factory waste- are of particular concern, especially because of their impact, which is currently being followed up. Although indirectly and in inconspicuous amounts, exposure to airborne metallic waste in factory effluents can cause some health problems if accidentally inhaled or exposed to air or food. Many researches have shown that heavy metals can cause respiratory problems, cancer, and even death (3-5).

Cadmium is one of the heavy metals found in factory waste (6). Cadmium comes from the air mixed with the results of air waste and water waste in equipment found in factory machines in chimneys. Cadmium is reported to have many cases of exposure from children to adults (7,8). Previous research states that the level of cadmium exposure is increasing every year for someone who lives around industrial areas. Cadmium exposure
causes various health problems such as cardiovascular disease\(^{(9)}\), impaired kidney function \(^{(10)}\) and itai-itai disease \(^{(11)}\).

Although evaluations continue to be carried out at a factory and the waste disposal regulations have been reviewed according to government policy standards, there are still cases of poisoning in someone who lives in an industrial area. Recent evidence suggests that adult people who live near industrial areas in the Amazon for more than 2 years have high cadmium levels \(^{(12)}\). The generalizability of much published research on this issue is problematic. However, habits and lifestyles have a strong relationship with a sufficient level of attitude and knowledge to address how to live in the industrial area. Up to now, far too little attention has been paid to know the correlation between knowledge, attitudes, and behavior correlated with blood cadmium levels. This paper attempts to show that basic factors can be a major cause for significant cadmium exposure. In this study, we focused on knowledge, attitudes, and behavior as basic factors of cadmium blood exposure.

### Materials and Methods

This study is a cross-sectional study design with a total sample of 108 people living in an industrial area. There are many criteria of inclusion such as: had a minimum of 1 year stay in an industrial area; distance of residence (within a radius of 5 km from industrial locations); and there are no respondents who had cardiovascular risk factor. The sample in this study was taken by purposive sampling from the study population living around an industrial area.

5 mL blood plasma from 108 respondents have been measured with atomic absorption spectrophotometry (AAS) in wavelength about 357.9 nm. Total cadmium content test method refers to the SNI 06-6989.17-2004 standard for testing metal content with AAS. The materials were obtained from the Laboratorium Terpadu Diponegoro University. A calibration curve is made to get the regression line equation then proceed with the measurement of the blood sample that has been prepared. Calculation of metal content is calculated using the equation below:

\[
\text{Total Concentration of Cadmium (mg/L)} = \text{Concentration} \times \text{fp (the dilution factor)}
\]

The questionnaire consisted of 10 questions to determine the level of knowledge, 15 questions to determine the level of attitude variables, and 10 questions for the level of behavioral factors (a total of 35 questions) distributed to 108 respondents (data not shown). The Cronbach’s Alpha value for all questionnaires is > 0.6 and all question items have a positive correlation ranging from 0.3 to 0.6 so it can be assumed that all of the questionnaire question items are valid and reliable. The totals of these statements were then used for further analysis using Pearson’s correlation. In this study, the relationship between the factors of attitude, behavior, and knowledge was correlated with cadmium data that had been done previously.

### Findings

The results obtained showed that the level of cadmium exposure was 82.41% which exceeded the normal threshold, namely > 50 ng/mL. The next result was followed by the normal category which was not much different from the abnormal category, namely 8.33% and 9.26%, respectively (Table 1).

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;5 ng/mL)</td>
<td>9</td>
<td>8.33</td>
</tr>
<tr>
<td>Not Normal (5.0 - 50 ng/mL)</td>
<td>10</td>
<td>9.26</td>
</tr>
<tr>
<td>Acute toxicity (&gt; 50 ng/mL)</td>
<td>89</td>
<td>82.41</td>
</tr>
</tbody>
</table>

In this study, we divided it into three levels for each factor. These factors include knowledge, attitudes and behavior. The results obtained from the data tabulation show that the knowledge that has a high level of respondents is 55.56%, the attitude has the highest proportion and 100% has a high attitude, then for behavior, it has a low level of 46.30 with the proportion highest in the observed population (Table 2).

### Table 2. Factors Category

<table>
<thead>
<tr>
<th>Variabels (N=108)</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
</tr>
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<tbody>
<tr>
<td>Knowledge</td>
<td>60</td>
<td>55</td>
<td>24</td>
</tr>
<tr>
<td>Attitude</td>
<td>108</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Behavior</td>
<td>30</td>
<td>27</td>
<td>28</td>
</tr>
</tbody>
</table>
In this study, shown in Table 3 are the results of research which indicate a significant relationship between the level of knowledge and cadmium levels ($r = -0.272$ *; $P = 0.047$) in the industrial area population. The attitude factor has a significant relationship with the knowledge factor ($r = 0.528$ **; $P = 0.000$) followed by a significant positive correlation between behavior and attitude ($r = 0.269$ *; $P = 0.049$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cadmium</th>
<th>Knowledge</th>
<th>Attitude</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>-0.272*</td>
<td>-0.23</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.047</td>
<td>0.095</td>
<td>0.932</td>
</tr>
<tr>
<td>Knowledge</td>
<td>r</td>
<td>1</td>
<td>0.528**</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.047</td>
<td>0.000</td>
<td>0.284</td>
</tr>
<tr>
<td>Attitude</td>
<td>r</td>
<td>-0.23</td>
<td>0.528**</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.095</td>
<td>0.000</td>
<td>0.269*</td>
</tr>
<tr>
<td>Behavior</td>
<td>r</td>
<td>0.012</td>
<td>0.148</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.932</td>
<td>0.284</td>
<td>0.049</td>
</tr>
</tbody>
</table>

**Discussion**

Cadmium (Cd) is a metal that can cause toxicity and is a risk factor for cardiovascular disorders. It was also stated that Cd is classified as toxic for humans and plants (13). In humans, exposure to Cd that is too high in the long term can cause various cardiovascular compilations including hypertension, as well as other diseases such as decreased kidney function and many other organ disorders (8). Cadmium has atomic number 48, atomic mass number 112, melting point 321 °C, and boiling point 765 °C is an element that is odorless or tasteless, and is very toxic (13). One of the mechanisms of action of cadmium is to cause oxidative stress (14). After entering the body, cadmium can replace the ion content in the body from the cytoplasm and membrane proteins such as ferritin which will reduce ion levels. These free ions will later experience oxidative stress through the Fenton reaction (15,16).

In industry, cadmium waste gets special attention, because metal compounds are a type of pollutant that has a high level of toxicity even though the exposure is at low concentrations (17). According to Yu et al (2010) (18), in a study conducted in the Hangzhou area, China stated that industrial areas have the highest level of Cd when compared to several other places such as residences and highways. This is consistent with the research we conducted where the cadmium contained in the respondents we studied was in a high range. Approximately 82.41% of the total population we studied had levels of exposure to blood cadmium that exceeded the toxicity threshold with acute toxicity levels, meaning that they exceeded 50 ng / mL. Cd found on a normal blood test is <5.0 ng / mL, with most results in the range 0.5 to 2.0 ng / mL (19). Lack of nitric oxide (NO) will cause decreased homeostasis of blood vessels which will lead to endothelial dysfunction (20). Impaired endothelial function will cause oxidation stress and will later become a factor in the occurrence of cardiovascular disease (21). This shows a high urgency because if it is not followed up immediately, it is feared that it will increase the level of exposure and the level of disease that is at risk of causing death. Previous research has shown that cadmium levels are reported to be significantly higher among residents in industrial estates than among non-exposed residents (22). Our study shows that knowledge is negatively correlated with knowledge with a correlation coefficient of -0.272. The results of the analysis indicate that the
increasing knowledge will have an effect on decreasing metal content and vice versa. According to Adebamowo (2006) (23), the domain factor that is very important for the formation of one’s actions is called knowledge. Our results are consistent with this statement, where there is a correlation between attitude and knowledge with a correlation coefficient of 0.528. This shows that with increased knowledge, it will be followed by an increase in attitude. The existence of this linear relationship has convinced us that knowledge is an important factor in improving attitudes in dealing with environmental pollution that occurs around industrial areas. With more attention to knowledge, it is hoped that cadmium levels will decrease(23).

Our results show no correlation between knowledge and behavior. Another research also shows that environmental knowledge does not have a significant direct effect on pro-environmental behavior(24). The study explains that although there is no significant relationship between knowledge and behavior, knowledge remains an important factor in determining a person’s attitude and behavior. Our research shows that there is a relationship between attitude and behavior with a correlation coefficient of 0.269. This relationship is classified as having a positive correlation, namely the higher the attitude, the higher the behavior factor. In other words, we found that knowledge does not have a direct impact on behavior, but that the awareness of attitudes will create a behavior that is aware of environmental pollution. In our research, cadmium metal exposure is more pronounced. This is in line with research conducted by Faize and Akhtar (2020)(25) which conducted studies on students regarding increasing knowledge and attitudes to the environment which can be done by providing further explanations with nature and providing understanding of scientific argumentation that increases knowledge and attitudes.

Conclusion

Our study shows a correlation between the knowledge factor and cadmium levels with a negative correlation, where the increased knowledge will cause a decrease in cadmium levels. The knowledge factor also has a positive relationship with the attitude factor, if increased knowledge is carried out it will improve attitudes. Attitude factors correlate with behavioral factors, so that if there is an increase in attitude as a result of increased knowledge, behavior can also increase. Therefore, from the results of our research, it is very important for the local government to evaluate the knowledge improvement of people living in industrial areas in an effort to reduce the impact of cadmium exposure and minimize cardiovascular risk due to long-term exposure. Increased knowledge can be in the form of increased education, further education on cadmium exposure, and can also be in the form of other programs to increase the knowledge factor. Thus, research on the knowledge factor of reducing cadmium levels can also be applied as suggestions for further research in the future.

Conflict of Interest: The authors declare there is no conflict of interest.

Source of Funding: This work was supported by the University of Brawijaya under Grant number 3905 in 2019 and also supported by Laboratorium Terpadu Universitas Diponegoro and World Class University (WCU) Program University of Brawijaya.

Ethical Clearance: This research was carried out when it had received ethical clearance from the UB Ethics Commission, Faculty of Medicine with number 014/EC/KEPK. Respondents included in the inclusion criteria determined in the study were given informed consent and a statement to be willing to participate in this study. If the respondent is not willing, it will be excluded and not included in the study.

References


Coronavirus :- An Emerging Pathogen

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Abstract

Corona virus disease -19(COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Primarily it causes respiratory illness in humans. The pandemic caused by the virus started in 2019 in Wuhan, China. Later it was declared as pandemic by WHO. In this article I am reviewing the coronavirus along with its microbiological structure, transmission, pathogenesis, clinical features and its management. The virus is a single stranded RNA virus. The virus enters the respiratory tract via mucous membrane of mouth or nose. It binds to ACE TYPE 2 receptor and causes alveolar edema and its destruction leading to decrease in gas exchange which further leads to hypoxaemia finally leading to acute respiratory distress syndrome (ARDS). It most patients COVID19 presents with mild symptoms and they doesn’t require any medical intervention. But patients presenting with severe symptoms needs vigilant medical attention and may require ventilatory support. Most common symptoms are fever, dry cough, shortness of breath, fatigue, nasal congestion. The infection spreads via droplet from an infected person. Infection with this virus may lead to severe inflammatory response leading to release of cytokines which causes increased capillary permeability and vasodilatation leading to hypotension which further leads to decrease in blood supply to multiple organs leading to multiple organ failure. There is increase chances of morbidity and mortality among elderly and in those with comorbidities. In elderly the chance of fatality is way more than younger people. No one drug has been found yet as the ultimate treatment of COVID-19. Many drugs are under clinical trial and researches are going on for vaccine as well. But until we get a definitive cure the only escape we have is prevention and the only means to do that is to practice social distancing, proper hand hygiene and sanitation.

Keywords:- SARS-CoV2, pathogenesis, transmission, ACE 2 receptor, alveolar edema, ARDS, prevention, Ground glass opacities, hydroxychloroquine, remdesivir.

Introduction

Coronavirus term is derived from corona that means crown like and this crown like morphology is seen on electron microscopy. This morphology is due to club shaped peplomers arising from the envelop.

Coronavirus is enveloped, single-stranded, positive-strand RNA virus. It is belongs to the order Nidovirales. This coronavirus family consists of pathogens for many animal species including humans. The recent pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2).1 After the rapid spread of coronavirus in various parts of the world, World Health Organisation (WHO) declared it as PANDEMIC.

During the start of corona virus infection it presents in a similar fashion like common cold and many times patient might be asymptomatic. Theryby they transmit the infection to others unknowingly via droplet spread. People of older age and people with comorbidities are at high risk of contacting the infection because of their weakened immune system and comorbidities their immune response is compromised which render them more susceptible. Most patients of younger age present with mild symptoms and do not need any as such medical attention foe their recovery, all they need to do is to self isolate themselves so as to brake the chain of transmission. But at the same time the elderly need...
medical assistance to survive.

**CORONAVIRUS LINEAGE:-**

**ORDER- Nidovirales**  
**FAMILY- coronaviridae**

The Coronavirinae and the Torovirinae are the two subfamilies of the family Coronaviridae. The coronavirinae is again divided into four genera: Alphacoronavirus, Betacoronavirus, Deltacoronavirus and Gammacoronavirus. Coronavirus is widespread and maximum variety of genotypes infect bats. But Alphacoronavirus and Betacoronavirus are subcategories which affects the humans.1,2,3

**MICROSCOPY:-**

Coronavirus is an enveloped virus. It has a large plus-strand RNA genome of size the 27-32 kb. It is capped and polyadenylated and its diameter is 80nm to 160nm. The Coronavirus genome confer a 5' cap and 3' poly-A tail. Numerous eminent surface projections are present, they cover the viral surface completely, this gives them the crown like appearance. The size of the surface projection is about 20 nm. 2/3rd of RNA of coronavirus codes for non structural proteins and rest of it code for essential structural protein. 1,3

**ESSENTIAL PROTEINS:-**

*Spike proteins-* The coronavirus spike protein is a type I glycoprotein that forms the peplomers on coronavirus particles. Coronavirus binds to specific cellular receptors through the spike protein. After the attachment of virus to the cellular receptor, the spike protein undergoes a conformational change which allows the fusion of viral membranes with the cellular membranes. The spike protein is vital for entry of virus, its cell-to-cell spread and for determination of tissue tropism. Spike is the major determinant of tropism and pathogenicity and is responsible for initiation of viral infection.

*Membrane protein-* This protein is present in abundance on the membrane. It plays a role in viral assembly and host interactions. Glycosylation of M proteins occurs for virus and host interaction. It can be N or O glycosylated.

*Envelop protein-* integral membrane protein. Plays important role in viral assembly.

*Hemagglutinin esterase(HE) -* HE glycoprotein is responsible for formation of second type of spike on group II coronavirus. The second type of spike is smaller and allows binding of virus with sialic acid on host cell surface glycoprotein.

*Nucleocapsid protein-* Is a phosphoprotein which binds with viral RNA and act as a regulator for viral RNA synthesis. So this protein is essential for transcription and pathogenesis.

*Replicase protein-* They can determine the rate of viral replication so it affects tropism and pathogenesis.

Spike protein is a glycoprotein which exhibits high grade of mutation whereas in rest of the structural proteins no significant mutation is seen in COVID-19. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) when compared with severe acute respiratory syndrome coronavirus (SARS-CoV) showed less preserved models of Spike protein.

Its incubation period ranges from 1 to 14 days and its clinical features resembles to that of SARS-CoV and MERS-CoV that is cough, cold, fever, dyspnea, chest tightness etc. SARS-CoV and COVID-19 shows alike clinical features because both of them binds to angiotensin converting enzyme 2 (ACE2) receptor in lungs.3,4

**EPIDEMIOLOGY:-**

*Origin – Zoonotic(most likely Bats).*

*Intermediate host - Humans.*

Spread of coronavirus occurs from one person to another via direct contact with an infected person or via droplet spread through coughing or sneezing.

The Reproductive ratio for COVID-19 is roughly between 2 to 4. Reproductive ratio is degree of transmissibility which denotes the no. of people that can get infected from a single infected person.

*Attack rate- 30- 40 %*

*Case fatality rate( CFR)- 3.4% world wide*
Timeline:-
- China notifies WHO on 2019-12-31
- First US case in Seattle 2020-1-15
- WHO declared pandemic on 2020-3-11
- National emergency on 2020-3-12

TRANSMISSION:-

It is a droplet infection, that is it spreads through tiny droplets released from nose and mouth of infected person when they cough or sneeze. The droplets can settle on the people around as well as on various surfaces. And the virus can spread if the virus enters the mucous membranes of mouth or nose of the person who comes in contact with those infected surfaces. For how long the viruses will survive on any surface depends on its type. Surfaces can be disinfected within 1 to 2 mins by using 62% to 71% alcohol rendering the virus inactive.1,5

Viability of coronavirus on different surfaces:-

- Airborne droplets-3hrs
- Porous surfaces(cardboard, paper, fabric)- upto 24hrs
- Hard, shiny surfaces(glass, countertops, plastic, satinless steel)- upto 72hrs

So it is very important to have as less contact as possible with surfaces which could have been in contact with many persons and to follow proper hand washing and sanitising techniques to decrease transmission by fomites. Despite the fact it is not possible to avoid touching all the surfaces so the only option there is to avoid touching the mucous membranes and to wash hands regularly. Alcohol based sanitisers and disinfectants can also be used to sanitise hands and surfaces respectively. There are few evidences suggesting feco-oral transmission. It has been found that stool might contain virus. And in such cases rectal swab comes positive

SARS-CoV2 is more transmissible than SARS-CoV, due to increased binding of spike proteins to ACE2 receptors as a result of mutation of spike protein.

PATHOGENESIS:-

SARS-CoV2 is a droplet infection. It means that it spreads via respiratory droplets which are released in the environment by coughing or sneezing. It can also spread via direct contact with an infected patient.

For binding of virus with host cell 2 receptors are needed ACE-2 receptor and TMPRSS-2(trans membrane protease serine precursor 2) receptor. After binding to these receptors membrane fusion and endocytosis occurs and endosome is formed. pH inside the endosome is acidic. Then uncoating of virus occurs and ssRNA is released. In the presence of chymotrypsin like protease, translation occurs leading to formation of RNA dependent RNA polymerase and non structural proteins (NSP). The RNA dependent RNA polymerase enters the nucleus via importin channel and forms multiple copies of ssRNA. The ssRNA comes out and assemble with the NSP and forms the complete virus. The virus comes out of the cell via exocytosis. This causes activation of macrophages which causes release of cytokines like IL-6, VEGF etc. This leads to organ damage.

Through the mucosal membranes viral agent enters inside the body of host then it enters inside the respiratory tract, travels there finally enters alveoli in lungs. in the alveoli type II pneumocytes are present, they carry a receptor for SARS-CoV2 that is angiotensin-converting enzyme 2 (ACE2) receptor. SARS-CoV2 binds with ACE2 receptor. The binding occurs because of high affinity of S glycoprotein present on the outer membrane of the virus towards ACE2 receptor. After the binding is complete endocytosis of virus occurs inside the cytoplasm of type II pneumocytes. Following endocytosis the lysosomal enzyme breaks down the lipid bilayer of the viral agent.

The ribosomes of the host cell is utilised by SARS-CoV2 for translation of mRNA into polyproteins which then forms the structural framework of virus.

Then initiation of self replication of polyproteins occurs which leads to an increase in the viral load in the patient. In the process of self replication SARS-CoV2 uses the RNA-dependent RNA polymerase present in the host. The polyproteins are then proteolysed by enzymes present in the host cell following which formation of various structural proteins like spike protein, E protein, nucleocapsid etc takes place. These proteins combines with the single stranded RNA which is formed by replication and forms the mature Virus.
The mature virus comes out of the type II pneumocytes by a process called budding. And during this budding the type II pneumocytes are destroyed. This destruction of type II pneumocytes causes release of specific inflammatory mediators which then leads to stimulation of macrophages. The stimulated macrophages in response release specific cytokines like IL-1, IL-6 and tissue necrosis factor-alpha (TNF-α). In case of acute inflammation IL-1 & IL-6 are released which causes fever. These specific cytokines enter the blood and causes contraction of the endothelial cells of blood vessels and they also causes smooth muscle dilatation. These two actions result in an increase in capillary permeability which further leads to leak of plasma into the interstitial spaces finally causing alveolar edema. The other major problem that occurs due to type II pneumocyte destruction is decrease in surfactant production. This is one of the primary functions of type II pneumocyte. The surfactant is the substance which maintains the surface tension and prevent the collapse of the alveoli. But due to decrease in surfactant production surface tension increases causing alveolar collapse. Due to the alveolar collapse there is :- Impairment of gas exchange. Due to this refractory hypoxaemia occurs.

There is an extensive increase in the work of breathing due to impaired gas exchange in an attempt to inspire as much air as possible against a collapsed alveolus as well as against interstitial edema. This mechanism leads to ARDS that is acute respiratory distress syndrome. Another feared complication alongside ARDS is disseminated intravascular coagulation. Disseminated intravascular coagulation (DIC) is what leads to the development of multi-organ failure.

During the destruction of type II pneumocytes inflammatory mediators are released they cause a neutrophil influx inside the alveolus. Neutrophils will destroy the virus by releasing reactive oxygen species and proteases. While destroying the virus the neutrophils causes destruction of all the alveolar cells as well.

Type I pneumocytes play an integral role in gas exchange so their destruction causes impairment of gas exchange. Type II pneumocytes produces surfactant so their destruction causes increase in surface tension which further leads to alveolar collapse. There is collection of fluid in the alveolus which consists of destroyed slough off cells and cellular debris that contains type I & II pneumocytes, macrophages & neutrophils resulting in formation of consolidation. The consolidation further interferes with the gas exchange, causing cough, dyspnea & hypoxemia. Hypoxaemia stimulates peripheral chemoreceptors causing the sympathetic stimulation leading to increase in rate of respiration and heart rate as a compensatory measure for decreased partial pressure of oxygen.

One of the most common complication seen here is systemic inflammatory response syndrome (SIRS). This is seen in case of progression of infection when the specific cytokines enters the vasculature causing increased capillary permeability. This increase in permeability of capillaries leads to leakage of plasma in the interstitial tissue spaces. This dumping of plasma in the extravascular space decreases the blood volume. Also the vasodilatation that happened due to the cytokines further decreases the total peripheral resistance (TPR). The decrease in both the blood volume and TPR leads to a considerable drop in blood pressure that is hypotension. This leads to insufficient perfusion of organs and this can anytime progress to multi-system organ failure. Every organ manifest in its own ways. Elevation of blood urea nitrogen (BUN) and creatinine is seen in c/o insufficient kidney perfusion. This elevation is seen because kidney is unable to filter the needed amount of BUN and creatinine from blood and this finally causes acute renal injury and renal failure. The circulating cytokines also enters the central nervous system CNS via the vascular system. The main site of action in CNS is hypothalamus. The IL 6 and IL 1 acts on the hypothalamus. Hypothalamus is the part of brain which maintains the body temperature. When IL-1 and IL-6 attains high concentration in hypothalamus release of prostaaglandins takes place and this disrupts the normal temperature of the body and resets the core body temperature to higher than normal resulting in fever. This means that one of the most universal and common symptom that is fever is caused due to elevated levels of IL-1 & IL-6.\textsuperscript{1,3,5,6,7}

**Clinical features:**

The duration between onset of symptoms and death varies from 6 to 41 days but it actually depends upon the age and immune status of the patient. The incubation
period is shorter among older individuals. India is still in better shape because the mortality rate is less compared to others.

Median timeline observed:

Day 1 – exposure to COVID-19

Day 5 – presentation of 1st symptom

Day 10 – dyspnea

Day 12 – hospital admission

Day 13 – ARDS

The people with comorbidities like asthma, diabetes and hypertension are at a higher risk. Other risk factors are patients on ACE inhibitors and ARBs as they cause upregulation of ACE-2 receptors. And this leads to an exponential increase in replication of the viral genome and it leads to increase in severity of the disease. And this also explains why people with hypertension are at a higher risk. This also explains the severity of infection in elderly as compared to younger people.

People at extreme of ages are at higher risk. Elderly people are at a comparatively higher risk because of the physiological change taking place in their lungs due to ageing. As age progresses the respiratory muscles get atrophied leading to reduction in respiratory functions. There is a significant decrease in lung capacity. Also the immune barrier becomes weak and the mucociliary clearance of the airway decreases. There is one more theory which suggests that with age no. of ACE2 receptors increases rendering the old people at higher risk of infection as compared to adults. Cardiovascular symptoms are also seen with COVID-19 patients. This is seen when the disease progresses and due to progression the immune system becomes defective. Myocardial injury also occurs due to cytokine storm as a result of imbalanced response between type 1 & 2 helper T cells.8,9

In the early phase the symptoms resembles common cold like cough, fever, cough and its difficult to differentiate but as the virus replicates and the viral load increases the severity of symptoms increase.

Damage to the alveolar epithelial cells causes fibrotic changes which leads to dyspnea, cough and hemoptysis. Multiple peripheral ground-glass opacities were observed in subpleural regions of both lungs in some patients on hospital admission. Every person gives a varied response depending upon his immune status. So the severity of symptoms and the fate of disease directly depends upon the state of immune system of patient. This explains why elderly, immunodeficient people and people with comorbidities show severe symptoms and higher mortality compared to those who are comparatively healthy with a stronger immune status.

ACE 2 receptors are also present on enterocytes, so gastrointestinal symptoms can also be present. Technically as coronavirus is enveloped so it shouldn’t have causes any GIT symptoms, but it does sometimes.

Generally in early stage the symptoms are not specific like fever, non productive cough, cold, bodyache, generalized weakness lethargy with or without diarrhea. Patient may also complaint of difficulty in breathing that is dyspnea. As the disease progress hypoxia may develop leading to ARDS (acute respiratory distress syndrome). In such situation mechanical ventilation is given to the patient in ICU. Patients spO2 is closely monitored. And in such cases the chance of secondary bacterial pneumonia increase. the commonly involved organisms are staphylococcus aureus, streptococcus pneumoniae, klebsiella pneumonia & hemophilus influenzae.

So the common symptoms are fever, dry cough, shortness of breath, myalgia, fatigue, sputum production, lymphopenia, nasal congestion, chills/night sweats.

Uncommon symptoms are headache, hemoptyis, diarrhea, confusion, rhinorrhea, chest pain, nausea, vomiting, sneezing.

Complications are Acute Respiratory Distress Syndrome (ARDS), secondary infection, ventilator associated pneumonia, septic shock, acute renal injury, hypoxemia, acute cardiac injury.8,9,10

EXTRAPULMONARY MANIFESTATIONS OF COVID-19:-

Neurologic – headache, dizziness, encephalopathy, ageusia, myalgia, anosmia, stroke

Renal – proteinuria, hematuria, acute kidney injury

Hepatic - elevated aminotransferases, elevated
bilirubin

Gastrointestinal- nausea, vomiting, diarrhea, abdominal pain, anorexia

Cardiac- myocardial injury, myocarditis, cardiac arrhythmias, cardiogenic shock, myocardial ischemia, acute cor pulmonale, cardiomyopathy

Endocrine – hyperglycemia, diabetic ketoacidosis

Dermatological – petechiae, livedo reticularis, erythematous rash, urticaria, vesicles.

BIOCHEMICAL CHANGES:-
- CBC- leukopenia & lymphopenia
- Increase BUN
- LFTs- increase AST/ALT
- Increase D-dimer, increase CRP, increase LDH
- Increase IL-6, increase ferritin
- Decrease procalcitonin

INVESTIGATIONS:-
-LAB DIAGNOSIS:
1) Antigen detection- coronavirus antigen in respiratory epithelial cell can be detected by ELISA using specific monoclonal antibody
2) Electron microscopy- shows morphology
3) RNA detection by RT-PCR:- can detect RNA in respiratory secretions.

RT-PCR: it stands for reverse transcriptase polymerase chain reaction. Via reverse transcriptase DNA is formed from the RNA of the virus and then it is amplified for detection.
4) Serum antibody detection by ELISA or hemagglutination.

- IMAGING:-
  a) Chest Xray: hazy bilateral peripheral opacities
  b) CT scan : ground glass opacities(GGO), crazy paving, consolidation.

CT scan is not done in every patient. It is indicated only in patient with moderate to severe symptoms and/or worsening respiratory status.

CO-RADS

Standardised reporting system based on CT findings is CO-RADS. It gives the level of suspicion.

<table>
<thead>
<tr>
<th>LEVEL OF SUSPICION</th>
<th>CT FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO-RADS 1</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>Normal or non-infectious abnormalities</td>
</tr>
<tr>
<td>CO-RADS 2</td>
<td>LOW</td>
</tr>
<tr>
<td></td>
<td>Abnormal findings consistent with infection other than COVID-19</td>
</tr>
<tr>
<td>CO-RADS 3</td>
<td>INDETERMINATE</td>
</tr>
<tr>
<td></td>
<td>Unclear whether COVID-19 is present</td>
</tr>
<tr>
<td>CO-RADS 4</td>
<td>HIGH</td>
</tr>
<tr>
<td></td>
<td>Abnormal findings suspicious of COVID-19</td>
</tr>
<tr>
<td>CO-RADS 5</td>
<td>VERY HIGH</td>
</tr>
<tr>
<td></td>
<td>Typical COVID-19</td>
</tr>
<tr>
<td>CO-RADS 6</td>
<td>PCR +</td>
</tr>
</tbody>
</table>
Typical appearance of COVID-19:- Peripheral bilateral ground glass opacities (GGO) with or without consolidation or visible interlobular lines (crazy paving). Multifocal GGO of rounded morphology with or without consolidation or visible interlobular lines (crazy paving). Reverse Halo sign (Atoll sign) or organising Pneumonia or spider web.

Indeterminate appearance:- multifocal, diffuse, peripheral or unilateral GGO with or without consolidation lacking a specific distribution and are non rounded and non peripheral. Very few small GOG.

Atypical appearance:- presence of isolated lobar or segmental consolidation without GGO. Discrete small nodules(centrilobular, tree in bud). Smooth interlobular septal thickening with pleural effusion.

Very atypical appearance:- cavitation, calcification, tree in bud, bronchiolitis, nodular pattern.\textsuperscript{11,12}

CT CHANGES OVER TIME:-

<table>
<thead>
<tr>
<th>DURATION (DAYS)</th>
<th>STAGE</th>
<th>CHANGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>Early</td>
<td>GGO, partial crazy paving, lower no. of lobes involved</td>
</tr>
<tr>
<td>5-9</td>
<td>Progressive</td>
<td>Extension of GGO, increase crazy paving</td>
</tr>
<tr>
<td>10-13</td>
<td>Peak stage</td>
<td>consolidation</td>
</tr>
<tr>
<td>14 or more</td>
<td>Absorption</td>
<td>Gradual resolution</td>
</tr>
</tbody>
</table>

CT severity score:- scoring based on the % of each of 5 lobe involved. Each lobe is given individual score and then sum is calculated.

- >5% involvement = score 1
- 5-25 % involvement = score 2
- 26-49% involvement = score 3
- 50-75% involvement = score 4
- >75% involvement = score 5

Score ranges from 0 (no involvement) to 25 (maximum involvement).

- if score <8 = mild
- if score 9 to 15 = moderate
- if score >15 = severe\textsuperscript{13}

MANAGEMENT OF COVID-19:-

Symptomatic management, supportive care and prevention of transmission forms the mainsaty of management. Social distancing is the most affective tool we have to break the chain of man to man transmission. Most patients presents with mild to moderate presentation ranging from mild symptoms to mild pneumonia. And the patient with mild symptoms recover on their own with any medical interference, all that is needed for them to do is to isolate themselves to break the chain of transmission. Vitamin c & d, zinc, selenium etc are being tried as measures to prevent from viral infections specifically respiratory.

Many drugs are under clinical trial. Hydroxychloroquine is being used widely in the treatment of COVID19 but there is no solid evidence to tell about its efficacy. And some studies even suggest that it is increasing the risk of cardiac arrest. REMDESIVIR is another drug under trail. Remdesivir inhibit viral replication. It is an adenosine analog. Multiple trials
are going on but no one drug has been narrowed down yet.\textsuperscript{14,15,16,17}

Drugs under trial are :-

- Hydroxychloroquine- inhibit entry of virus by inhibiting ACE-2 & TMPRSS-2 receptor, inhibit endocytosis by increasing pH inside endosome.
- Nafamostat – TMPRSS-2 blocker
- Lopinavir , Ritonavir- protease inhibitor
- Remdesivir , Ribavirin, Favipiravir- RNA polymerase inhibitor
- Ivermectin – importin inhibitor
- Immunomodulators
  a. corticosteroids, hydroxychloroquine
  b. anti IL-6 – tocilizumab, sarilumab
  c. anti VEGF- bevacizumab
  d. plasma exchange

Control measures:-
- isolation of patient
- quarantine of exposed people
- travel restrictions if needed
- personal protective equipment- gloves, gowns, goggles etc.
- frequent hand washing with soap and water
- use of alcohol based sanitizer
- sanitization of surfaces which come in direct contact
- use of masks and shields at public places
- avoid personal contact with sick patient\textsuperscript{18,19,20}

Conclusion

This is a review article on coronavirus in which I have dealt with its basic morphology, transmission ,pathogenesis , common clinical features and management. This is an emerging pathogen , not much information is present regarding this virus. It is primarily causes respiratory infection and spreads via droplet from person to person. Mostly is causes mild symptoms like cough , fever, cold , headache, loss of taste and smell. But in severe cases is can cause dyspnea , ARDS, pneumonia and even multi organ failure. It attaches to ACE 2 receptor and causes fluid dysregulation in the respiratory tract leading to alveolar edema leading to dyspnea. On CT scan ground glass opacities are a consistent finding. Mutiple drugs are under trial but no one drug has been proven 100% affective. Drugs like hydroxychloroquine, ivermectin , remdesivir etc are being used. Still the best remedy remains social distancing, sanitising and using personal protective equipment.

Material and Method

This is a review article . various articles have been reviewed.

Acknowledgement:- This work is partially supported by the DMIMS and further more information is collected from various journals .

Conflict of Interest- Nil

Funding- DMIMS(DU), WARDHA

Ethical Approval- From Institutional Ethical committee, DMIMS

References


Role of Ayurveda in the Era of COVID-19

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Abstract

Summary: Currently, we can see, covid19 is quite a concern because of its mortality and even morbidity rate. Since no cure is present till date it’s more important for people to take basic care to increase their immunity. One modality for prevention of such diseases can be through Ayurveda, which has different therapies and medicines to deal with diseases as well as measures to take basic care and to boost immunity of individuals. AYUSH helps with those basic methods in one’s life. This article discusses the necessity of ayurveda in order to cure this type of air born diseases. Ayurveda mainly emphasizes on body response to any cure as well as role of chakra healing in covid19. Susruta helps find chakras in our body and chakra helps to communicate with different diseases via factors like air, water, earth, fire etc. Ayurveda is the oldest cure and best way to connect body with mind and soul. It brings you more near to nature and helps you calm yourself in different situations. It also molds your perspective and the way we see things.

Conclusion: Lifestyle adaptation with proper dincharya and ritucharya is quite effective to ensure healthy living and protect ourselves by building our immunity. Ayurveda acts as an immunity booster and effective in combating infectious diseases.

Keywords: Janapadodwasma (Covid19), ayurveda, herbs, dincharya and ritucharya in covid19.

Introduction

Janapadodwasma and covid19

The world is terrified due to pandemic corona virus disease (COVID-19) which is mainly caused by severe acute respiratory syndrome Corona virus (SARS-CoV-2). This is considered as Janapadodwasma (destructions of states or kingdoms or country) in ayurveda. Moreover COVID-19 infection may be correlated with Vata-Kaphaja Sannipataja Jwara (a type of fever mentioned in classical Ayurvedic texts with severe complications and and problems to one’s living) (¹). Thus we have to understanding the concept of COVID-19 and its management principles based on ethics of Ayurveda. The main aim of management principle includes correction of the Vikruta (contaminated) Vaya (air) and Desha (place/continent etc.) and improving the immunity and lifestyle for prevention of disease as well as the management of COVID 19 patients by various herbal or herbo-mineral combinations which is mainly based on the stage/severity of the disease along with which patients are asked for follow up for recovery to avoid the recurrence. In this aspect the management of COVID-19, role of Ayurveda experiments are proved more beneficial in asymptomatic, mild and moderate stages. Further, clinical studies on these drugs should be conducted to make sure evidence for safety and efficacy on COVID-19 for wider acceptance and implementation of Ahara, Vidhis, Dinacharya and Sadvritta in National Health Policies for improving disease resistance. In ayurveda it is termed as Janapudhwamsa; in Caraka Samhita its concept has already been described, Caraka Samhita being a nodal text helps you to understand it better and it is also correlated with pandemics and epidemics too (²,³). According to this janapudhwamsa is classified into two group based on their causes; (⁴,⁵)

- First- occurring due to natural causes
- Second- occurring due to manmade causes.

All two of them mentioned above are further classified into four groups that is:

1. Vitiation of water
2. Vitiation of climate
3. Vitiation of land and
4. Vitiation of air

Etiology of janapdodhwamsa

**Water:**

Water is considered to be harmful (vitiating) when –

1. Water has different texture, taste, color, odor or smell,
2. Water that is muddy and is turbid and
3. The water which doesn’t have any type of aquatic animals in it.

**Climate:**

Some Features of destruction or vitiation of climate includes-

1. Unseasonal climate variations,
2. Regular climatic agitation, earthquakes, meteorites, etc and
3. Presence of poisonous fumes that veil the skies.

**Land:**

Land is considered to be problematic or vitiating when-

It is rife with grass or weed, withered and dry, major of forest resources are damaged and are excessively moist – like it has no predatory animals like birds, vultures, jackals, serpents and carnivores, a bog or marsh and is riddled with smoke and Dust.

**Air:**

Vitiation of air is considered when –

Air is constant without any movement, turbulent and fear full, like moist, hot, dry and blowing in opposite directions frequently, huge cyclones, harmful and toxic gases in it etc.

These all are responsible for violation of climate, water, air, land and finally leading to epidemic and pandemic. Now since we are clear about, how janapdodhwamsa work, there are some ways to understand the spread of air born infection in ayurveda.

**Comprehending infectious diseases (air born) in Ayurveda**

One of the terms being regularly used is “Bhuta” which can be found in various scripts in ayurveda .one of the example of this is the text of Panchamahabhootas (6) – where they have explained the foundation and basis of the microorganism and the macro organism of this world. Panchamabhootas has been used for all microorganisms commonly known as ‘Jeevanus’. The character that microorganisms or microbes play in manifestation of disease has never been hidden and known since the time of Vedas. , Krimi (worms that leaves inside human body), Bhuta (ghosts), Jantu (creatures), Rakshas (demons), Jeevanu (bacteria), and Pishacha (vampires) these terms have been used to describe them. The “Panchamabhootas” are therefore classified on the basis of visibility and on the basis of infection(4,5). Thus, spread of these diseases is main concern to deal with.

**Transmission of infectious diseases**

In ayurveda one of the renowned personality Susruta has describes that the manner of spreading of infectious diseases is mainly through contact with an diseased person frequently, food sharing and having physical contact via respiratory droplets , using materials such as combs, napkins, towels, blankets, bed sheets, etc. and exchange of body fluids in terms of sexual contact. Interestingly in ayurveda they all have cure for these spreads but before knowing, how to control them, one should know how Ayurvedic medicine work with pathogens in our body. Thus to continue further lets first understand the basic concept of approaching Ayurvedic medicine in individuals.

**Pathogenesis of infectious diseases and Ayurvedic approach**

There is no as such description describing how pathogens work and what the basic of occurrence of those pathogens is. There are diseases like Agantuj Jwara (external factors leading to fever), Agantuja Atisara (Diarrhea), Ajirna (indigestion due to infection), Visuchika (Cholera leading to diarrhea), etc are some scenarios where vitiation of Doshas take place which occur mainly because of infectious diseases, this can be
related with the infectious agents. In rest of the scenario or cases, no different descriptions are available for this type of infectious diseases and are usually known by the type of infectious diseases.

Time of Disease origin, its development and progression of any infection is written in the description in Shat Kriya Kala - here one thing is important to note that according to the line the main agenda of ayurveda is obtaining this stage of Dhatus Samya – (that is equilibrium of Dhatus) (3) and to study about these things that being grasping the knowledge of Hetu (refers to etiological factors), Aaushadha (Drugs or medicines) and Linga (clinical presentation)(4) for healthy person as well as diseases person its important to free them from these pathogens so that there healthy life could be maintained and the person is treated in order to stabilizes his efficiencies back. In pathogenesis according to above mentioned stages the progression of disease is being assessed (5) and thus they are considered as the stage of treatment. This mainly includes corrupting the situation of Malas, Dhatus and Dosha.

- First stage - Dhatu
- Second stage – Dhatu and Malas
- Last stage and third stage- differentiation or complication

In first three stages there is lodgment of infective agents which are disturbing the Dhatas. The bodily elements are spoiled by factors such as environment, diet, indulgence, modification of lifestyle.

In the last stage of pathogenesis there is migration and circulation. It is caused by the Vayu by the means of Rasa (blood circulation, lymphatics, interstitial fluid, etc) (6). these circulating Doshas accumulate in places where it doesn’t affect or temper the body element (6,7). It means that, the knowledge of infection is good to Ayurvedic scholars.

How dincharya and ritucharya will help in Covid-19?

“Ayurveda extensively describes many ways on preventive care through Dinacharya and Ritucharya, which means daily and seasonal regimens. It is basically a plant-based science and improving immunity has been emphasized in many number of ways in Ayurveda literature,” says senior Ayurveda specialist Dr S Sarangapani. (8,9)

Dincharya helps u to emphasize on things that we were ignoring since long time. It’s a high time one should make a proper timetable to spend their day more efficiently. Do yoga, eat healthy, talk to your elders, be good to people, try to find your weakness and way out of it, learn new things, and use the time to grow every day. Yoga bring u peace, it cures your body help u to analyze things more clearly. It’s a food to your soul.

While ritucharya is more Ayurvedic secrets for seasonal eating. Where ritu stands for seasons and charya stands for guidelines. Our body is designed in specific manner which mainly help us to work with different natural conditions including different seasons. These guidelines help us to follow a proper diet and lifestyle which include detox treatment.

According to ayurveda year is mainly divided into two time periods - uttrayana (cold climate) and dakshinayana (hot climate). Uttrayana further is divided into - season of sharath, hemanta, and shishira. While dakshinayana is divided into season of vasant, grishma, and varsha. (9,10,11)

Thus eating according to seasons helps built immunity and prepare body to fight with any seasonal infection that harms your body. There is two pictures that help you with the dincharya and ritucharya understanding.
COVID-19
Coronavirus Disease 2019

10 WAYS YOU CAN HELP THE ELDERLY AND PEOPLE WITH UNDERLYING CONDITIONS LIVING WITH YOU

Monitor their health for symptoms of COVID-19, like fever, cough, and difficulty breathing.

If anyone in the household has symptoms, avoid physical contact and take heightened prevention measures.

If they have COVID-19 symptoms, contact a healthcare provider immediately.

Encourage them to maintain a healthy lifestyle: eat nutritious foods, get enough sleep, don’t smoke, stay active and limit alcohol use.

Regularly clean and disinfect surfaces and keep the house well ventilated.

Avoid sharing objects like glasses, cutlery and towels.

Make sure they have at least a month’s stock of all their regular medicines in case it’s necessary to stay home. Encourage them to follow medical advice about medication.

Follow social distancing measures in your area and avoid shared spaces and large gatherings.

If going to the doctor, wear a mask, if available, and avoid public transport if possible.

Be kind and show empathy. Talk and listen to them. Help them cope with stress.

Info graphic: COVID-19. Ways you can help the elderly and people with underlying conditions living with you.
Boosting Immunity

HOW TO MAKE AYURVEDIC HAND SANITIZER AT HOME

herbal sanitizer comprise of combination of alcoholic extracts of Ocimum sanctum (Tulsi), Azadiractindica (Neem) and Eucalyptus globulus (Nilgiri) using suitable excipients; which can be used as a ready-made herbal hand sanitizer.

Even if Ocimum is not available one can use nail paint remover with aloe vera gel and rosewater for making hand sanitizer.

Herbal prevention for boosting immunity in covid19 pandemic

It’s important now to take care of ourselves and young ones and elders in this pandemic.

The best Ayurvedic medications which are affective and economic can be found very easily in households we usually use it in our daily basis. Some of them are mentioned below with their properties.

Ginger used for cold and cough

Ginger is very effective home remedy for cold and cough. Ginger root have anti-inflammatory properties, ginger and shogal can help relieve sore throat, reduce congestion and soothe an upset stomach. These compounds can also kill rhinoceroses (nasal wall thickening and secretion), which cause colds. Make ginger tea with honey and drink it three or more cups per day until you feel good this help you to cure cold and cough.11

Facial steam good for Runny Nose

It is already a traditional to take hot steam to treat runny nose. Steam cleanses your nostrils and helps relieve mucus and blocks caused by it. How to do it?
– Place your face over steam water, cover with cloth or towel to make sure no steam goes out and breathe deeply through your nose. Do this for 20 to 30 minutes at a time twice a day. Make sure you take a break if your face becomes too hot. Then inflate your nose to get

Figure1: immunity boosters in covid, By M. Sai Gopal
rid of mucus (don’t try too harshly). You can also add a few drops of decongestant essential oils (like Vicks, eucalyptus oil) to your facial steam water. Eating spicy foods also helps prevent runny nose and unblock nostrils (12).

Gargle with warm, salty water to treat any sore throat

Sore throat is very common, they can be mild or can be severe leading to voice getting worse though they can even get better than themselves within a week. A sore throat is mainly and majority of time is caused by a virus (such as a cold or flu) or smoking. Its Very rare that they can be caused by bacteria. If you are also suffering from a sore throat, gargle with warm, salty water for at least 5 minutes thrice a day(13).

Drink lots of water if diarrhea is the problem

Many a times, diarrhea resolves on its own. This condition also can be caused by various factors including viruses, bacteria, parasites, food intolerance and digestive disorders. If it’s Diarrhea, you need to quickly take fluids to treat dehydration. So, drink plenty of water, Taking probiotics, eating green bananas can also help reduce the symptoms of diarrhea. Chamomile and peppermint are also useful to treat diarrhea(14).

At last role of dhoopana in ayurveda

Dhoopana is a method by which drugs of herbal, herbo-mineral or animal origin are used for fumigation so as to heal Varna (words), Yonivyapada (vaginal disorders), Karma Rogas (ear diseases), Nasa Rogas (arterial diseases), Guda Rogas (arterial diseases), Gatra Daurgandhya; to disinfect Bheshajagara, Vranagara, Sutikagara, Shastrakar-maghruha, and Kumaragara and also to sterilize Asavas and Aristas (15,16,17).

Ayurveda has a broader scope for improving immunity and combating infections like Covid-19 (18,19,20). A number of ayurvedic herbal drugs are being trialed as antiviral herbals in Covid-19 (21,22).

Conclusion

So it is been clear that apart from well known medical help from ayurveda in this pandemic ,importance of it have been declined, in spite of the fact that it plays a major role in peoples life by boosting their immunity.

In ayurveda According to Charaka (renowned Ayurvedic practitioner) – where he said that disease is the combination of two things namely which he named it as Dosha (3 substances present in human body –vata, pitta, and kapha) and Dhatus (metals) and the gifted qualities of Doshas are more dominating over Dhatus. This proves that our body responds quickly after getting affected by pathogens regularly.

If our body is infected it can easily be treated through this methods. Though it has already been seen that not every time herbs help to heal patients and fails to prove its value for treatment of microorganism, but it is well known that ayurveda have always helped one to boost immunity and make individuals body more stronger against microorganism, so these herbs or medicine are tend to increase body’s action against diseases

Before this, the mentioned herbs should be the prime initiative for one’s to deal with this covid19 situation. Its more effective and economical. There are many articles that are available on this topic the more u read the more u can help yourself to protect from this pandemic. Ayurveda is just most basic form to hold your roots; there is always a reason that our ancestors followed this culture of dincharya and ritucharya. Meditation, eating healthy, suryanamaskar, early to bed , early to wake up, helps to form a clock (alarm) to people body that make their organ work in a systematic manner.

Concluding to this, Ayurveda might have not produced cure for this pandemic problem, but its been proven that it for sure helps people to boost their immunity against covid19.

Conflict of Interest: Nil

Funding: DMIMS (DU), Wardha

Ethical Approval: From Institutional Ethical Committee, DMIMS.

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Engaging School Going Children During Covid-19 Lockdown

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Abstract

Corona virus (Covid-19) is a pandemic disease, it is a crisis situation faced all over the world. The corona virus originated in bats and was transmitted to humans in the month of December 2019 from Wuhan China. Corona virus is the infectious disease and it attacks on respiratory system and causes respiratory illness that can spread from one person to another person by droplets i.e sneezing, coughing. In India, as on date recorded Corona virus cases are 207615, recovered cases are 1,00303, total deaths 5815 as on 30 August 2020. Indian Government has declared lockdown all over the states to save the life of the citizens. This is a very critical situation mostly for vulnerable population means children. After lockdown children are getting bored, due to separation from friends, peer group and their daily routine of playing is creating lots of anxiety for children. Children’s social life and leaving have been affected. Lockdown of pandemic disease, Closure of schools has affected the education of children and younger’s also. The school closures are liable to boredom to the students and caused learning gap among the children belonging to lower or higher socioeconomic status families. In this condition we have to understand the children psychology and we need to engage them in creative and interesting activities at home, also need to give them some learning task regarding studies so their cognitive and psychomotor function will be improved. This short communication concludes with the key learning for the parents of children on how to engage school going children during lockdown in Covid 19(Pandemic).

Keywords: Covid-19, Pandemic, Lockdown, Engaging, School kids.

Introduction

Corona viruses are a large family of viruses which may cause illness in animals or humans. In humans, corona viruses causes respiratory illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome [1]. It is an infectious disease and can be spread through droplets from one person to another person those in contact with a person infected with corona virus [1]. The World Health Organization has officially declared COVID-19 to be a pandemic and has called on “all countries to continue efforts that have been successful in reducing the number of cases and halting the spread of corona virus infection.” The primary purpose of Lockdown is to stay at home and to prevent spread of corona virus in community and maintain communal space [2]. Lockdown has started in phase. The 1st phase was from 25th March 2020 to 14th April 2020 for 21 days, 2nd phase from 15th April 2020 to 3th May 2020 for 19th days and 3rd Phase from 4th May 2020 to 31st May 2020. In this crisis, children’s are the vulnerable population they are physically and psychologically disturbed due to disturbances in their daily routine and separation from their friends and peer group, closed relatives. Parents are worried how to engage their children during lockdown [3].

EFFECTS OF LOCKDOWN ON SCHOOL GOING CHILDREN

During this Covid 19 pandemic disease children are luckily safe because of early decision of school closure by state government and central government but it has made profound effects on children’s well being. Lockdown make more adverse impact on children
belongs to lower socioeconomic status as it is widening learning gap because access to mid-day meals is the primary reason so many Indian students attend school, if the lockdown continues for much longer, there is a chance that India’s dropout rate—which is already among the world’s highest—might increase further[4,5].

According to United Nations Educational, Scientific and Cultural Organization (UNESCO), about 0.32 billion students in India have been affected because of school closures due to the Covid-19 pandemic (UNESCO 2020). Of these, almost 84% reside in rural areas while 70% attend government schools. As of 2015, the average dropout rate across secondary schools in India was 17.06% with higher numbers for rural areas [6].

Although parents having access to technology are gradually switching to online education for the studies of their children while others are still not able to do so. It is important to provide practical support to parents and caregivers, including how to talk about the pandemic with children, how to manage their own mental health and the mental health of their children and tools to help support their children’s learning. Now is the time to step up international solidarity for children and humanity and to lay the foundations for a deeper transformation of the way we nurture and invest in our world’s youngest generation [3,7].

Although children are not the face of this pandemic, they risk being among its biggest victims. From socioeconomic impacts to physical and mental effects due to mitigation efforts, the corona virus pandemic is a universal crisis, that for some children, the impact will be lifelong.

**NEEDS OF SCHOOL GOING KIDS DURING LOCKDOWN.**

The school going children have different types of needs like emotional and physical needs. The emotional needs are like love, affection, need of comfort. The physical needs of school going children are safety, security, warmth, comfort, trust, food, clothing, safe drinking water, fresh healthy air, a secure surrounding environment, time for indoor and outdoor games, adequate amount of quality sleep and rest. In this period working parents are getting adequate amount of time to care for their children because of work from home due to lockdown. It’s a good opportunity for parents to see their children in growing stage closely.

Along with all needs education is also must for children, it’s a part of their future and they are future of tomorrow. So we cannot separate them from studies, parents has a greater responsibility belonging to lower or higher socioeconomic status to help their children to impart the knowledge. So their cognitive function will be improved. The school is important for such kids as they not only learn but also get therapy sessions. Plus, there is a dedicated person working with each child [8].

**HERE ARE A FEW CREATIVE AND INTERESTING ACTIVITIES SCHEDULE TO KEEP KIDS BUSY DURING LOCKDOWN [3].**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-7am</td>
<td>Wake up early in the morning. Do Activities of daily leaving (ADL) and Do exercise, meditation and pranayama with all family members.</td>
<td>It will help to maintain the health and boost the immunity of all family members and can fight with corona virus infection.</td>
</tr>
<tr>
<td>7-7.30am</td>
<td>Healthy breakfast</td>
<td>Healthy living and boost the immunity</td>
</tr>
<tr>
<td>7.30-8am</td>
<td>Do the bath</td>
<td>Maintain personal hygiene</td>
</tr>
</tbody>
</table>
### Cont... HERE ARE A FEW CREATIVE AND INTERESTING ACTIVITIES SCHEDULE TO KEEP KIDS BUSY DURING LOCKDOWN [3].

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-10am</td>
<td>Participate in family chores</td>
<td>To know the sense of responsibility</td>
</tr>
<tr>
<td>10-10.15am</td>
<td>Do worship and prayer as per religion</td>
<td>Maintain the spiritual need of the family members and create positivity.</td>
</tr>
<tr>
<td>10.15am-11am</td>
<td>Playing indoor game</td>
<td>To engage the children and for their entertainment during lockdown.</td>
</tr>
<tr>
<td>11-12noon</td>
<td>Make creative and constructive things.</td>
<td>To engage the children during lockdown and increase the critical thinking.</td>
</tr>
<tr>
<td>12-1pm</td>
<td>Have healthy lunch</td>
<td>To maintain good health</td>
</tr>
<tr>
<td>1-2pm</td>
<td>Playing cards, chess, carom, ludo game with all family members.</td>
<td>To maintain good interpersonal relationship.</td>
</tr>
<tr>
<td>2-3pm</td>
<td>Rest and sleep</td>
<td>To maintain good health</td>
</tr>
<tr>
<td>3-4pm</td>
<td>Do school assignment which has been sent by teachers on Whatsapp group or mail.</td>
<td>To keep in touch with academic curriculum and studies.</td>
</tr>
<tr>
<td>4-5pm</td>
<td>Snacks and milk or tea</td>
<td>To preserve good health</td>
</tr>
<tr>
<td>5-6pm</td>
<td>Playing physical activity game like badminton, table tennis.</td>
<td>To maintain the health and entertainment also.</td>
</tr>
<tr>
<td>6-7pm</td>
<td>Watching TV</td>
<td>For fun and entertainment</td>
</tr>
<tr>
<td>7-8pm</td>
<td>Dinner</td>
<td>To maintain the health</td>
</tr>
<tr>
<td>8-10pm</td>
<td>Playing with family members like antakshari making dance videos etc.</td>
<td>For fun and entertainment</td>
</tr>
<tr>
<td>10pm-6am</td>
<td>Drink one glass of lukewarm milk, rest and sleep.</td>
<td>To maintain good health</td>
</tr>
</tbody>
</table>
Plan Daily Activities During Lockdown

1. Practice and teach yoga:-

It’s a good chance for the parents to teach Yoga to their kids during this lockdown at home. Otherwise busy parents are free during lockdown hence this time can be utilized for teaching yoga to their kids. Yoga helps in improving health and can also increase their immunity power and make them stronger. This lockdown can also be used to make kids understand the importance of good health and to teach them some healthy habits. Things learned during this young age of their life will remain with them forever and will help them to become fit throughout their life [9].

2. Arrange cooking class at Home

Lockdown is very good opportunity to teach your kids some easy to make recipes at home, by this they will come to know how food is made and what are the ingredients required to prepare food. This habit will help them in future too, like if they are going out for some higher studies or so all alone, then in such case they can cook for themselves and for others too and can become self dependant [9].

3. Try to teach them new languages

Many scholars in the history were well versed with more than one language; it helped them to understand literature of different countries and helped them to gain more knowledge. In today’s generation also knowing multiple languages is an additional benefit. In fact for taking admissions in various foreign universities you should be aware of their local language by considering all these things this lockdown period can be utilized for teaching or ask them for teaching new languages. You can use online available sources for the same or can also use various You Tube channels for teaching your kids. As whole family is at home kids can learn and practice new things with family members also [9].

4. Teach your kids art and craft

You can involve your kids in art and craft, this will help them to learn new things and they will remain busy during lockdown, it will also increase their creative level. You can teach them to make easy, simple craft projects which will help them to grow and show their artistic talent. This activity will help them in free from stress, tension and irritability due to lockdown period. You can also teach your kids how to prepare wooden or cloths puppets toys in a very easy manner at home, how to make simple ornaments by using various things at home [9, 10].

Following is the online arts activity taken by a private school during lockdown [10].

5. You can teach kids - art of writing letters

Writing letters in various formats is also an art you can teach your kids during this lockdown at home. Writing on wooden things, or mixing letters in an artistic manner can be taught during this period of lockdown. By this kids can learn how letters can be written in different formats; they may create some new formats of letter writing and most importantly it will help them to improve their handwriting [9, 10].

Following is an example:
6. Engage with Lego and puzzles games

Lego toys are so popular among children, in this game they can pass time and at the same time they can learn some basic things of physics. You can start with simple structures and can slowly move towards more critical steps with respect to your kids understanding [9].

7. Make Plan a scavenger hunt

In simple words it is called ‘ Luka Chhippi’ hide some things in your house at different places without knowledge of your kids and ask them to find it out, to make this game more interesting you can give them some clues. This will help kids in using their brain and will help them in developing their brain. They will also improve their thinking capacity [9].

8. Tree Plantation and Surrounding Cleaning

You also can teach your children importance of trees and plants in our lives, how they provide oxygen and their importance for Rain in the country. A small tree plantation drive at home will work for this; children will get connected with the soil also. During this break we can also teach children’s the importance of cleaning in life, how it is important with respect to stop spreading diseases. A daily morning cleaning drive around and inside the house will help them to understand the meaning and importance of cleaning; collecting garbage and dumping it into the dust bin will also help them in becoming a good citizen. We can also teach them how some specific in-house garbage can be converted into compost, we can teach them practically how compost can be make in-house and how it is better than readymade compost available in the market and how it is beneficial for the development of the soil.

9. Have an indoor picnic:

In covid19 nobody will allow children to go outside and play outdoor games according to their choice, as a result again they will became bore, we can handle this situation by arranging small picnic at home for children. Grab a sheet and whatever food children have, let them enjoy a living room picnic. Identify some indoor innovative games, which they will enjoy along with other family members during this in-house picnic, it will become helpful in engaging children and will also keep them refreshing time by time.

10. Story telling

In this period of lockdown telling inspirational, motivational stories can also help parents for engaging their children. It has both benefits as it will keep them engaged and also they will learn new things. Grandparents in this regards are the best story tellers, they should take interest for telling various stories to their grandkids. Story reading can also be useful as it will help in continuing their reading habits and will also learn new things. If stories having various characters they voice over can also be used for each character, which will keep children more engaged in stories. We can also provide them with different story books like pictorial story books etc, we can also engage them by playing stories on you tube or by playing audio CDs on our players and can ask them to listen carefully, this will make them a good listener too.

11. Play with the cat or other domestic animals

Some families are very much fond of cats or other domestic animals like dogs, parrots etc. We can teach children about importance of these animals in lifecycle, we can also teach them as how to take care of these animals, how to feed them. As a result children will also start loving and caring these animals. This will help them grow in a better loving and caring person.

12. Wash your car and two wheelers

Almost every family these days is having four wheelers at their place. This generation kids are very much interested in these machines. This interested of kids can be utilized to overcome boredom during this period of lockdown. We can teach them how to clean the car from inside, how to wash it with solutions, we can also introduce them to the small parts of the vehicle, also we can teach them how can particularly runs or about important documents required for having a car at home. This will help them to find out their own solutions during the times of emergency and will also help in engaging them during lockdown.

13. Singing and Making a song and dance

Singing and dancing are very important with respect to relaxing our body and mind. Making or writing new songs and converting them into the tunes can become a great engagement activity for kids during this period of
lockdown. Encourage your kids to write songs in their own words, later ask them to mold the same in beautiful tune, this will improve their writing skill, will also force their brain to think in different manner. We can simply engage them by playing dance songs at home and ask them to dance as per their wish, if you are good at dance you can teach them different steps.

Quarantine means isolation it does not mean just getting bored at home. There are various activities that can be arranged for killing boredom of your child, for keeping them engaged and to feed them physically as well as mentally. Stay home, stay positive and enjoy much needed rest with your family!

As a parent, you can utilize this time to DEVELOP GOOD HABITS in children at home during Covid 19 pandemic.

How to deal with a defiant child as a parent?

Understand what really going on

Decision fatigue: Giving them as many choices as you can

Treat them like an adult

Instead of giving them chorus, give them problems to solve

Ask their opinions and give them some control over their lives

Discussion

The present article is related to Covid 19 pandemic for short communication, it’s very difficult period for all. But in this period most vulnerable population in society are kids. They are getting bored so as parents it is our responsibility to engage them with various enjoyable activities like indoor games. We can plan various activities to keep them physically and mentally engaged. It will help them grow normally and free from stress and tension. It’s a good chance to explore their hidden talent. They can spend their whole day very nicely with creational activities. In this period we have to treat them like matured kids to encourage them so that they will do all activities whatever told to them and we will get the required output out of these activities.

Conclusion

Schools are closed due to the COVID-19 pandemic outbreak for an uncertain period of time, parents face the daunting task of figuring out how to help their kids learning and more likely how to keep them enjoyable and free from psychological health issues and physical health issues, while social distancing is either required or encouraged. It has become important than ever to make time to sit with their kids and plan their day. We all know that keeping positive feelings and thinking promotes well-being and community and parents may plan to schedule game nights, face time calls with relatives, friends for their children and include them in activities they find fascinating.

Acknowledgement

The author thanks to Dr.Seema Singh, Professor, cum Principal, Smt.Radhikabai Meghe Memorial College of Nursing, Datta Meghe Institute of Medical Sciences, (Deemed to be university) Sawangi (Meghe) Wardha, Maharashtra, India. For her timely support and valuable suggestions. I am very thankful to Mrs. Jaya Gawai, Dean Academic, Smt. Radhikabai Meghe Memorial College of Nursing. Datta Meghe Institute of Medical Sciences, (Deemed to be university) Sawangi (Meghe) Wardha, Maharashtra, India. For her valuable suggestions. Special thanks to Dr. Amol Bhawane, Assistant Professor, Medicine and Nephrology unit, Acharya Vinoba Bhave Rural Hospital, DMIMS (DU), Sawangi (Meghe), Wardha, Maharashtra India. Authors are also grateful to authors / editors / publishers of all those articles, journals and books, from where the literature for this article has been reviewed and discussed. The Authors are grateful to medico legal update editorial board members and medico legal update team of reviewers who have helped to bring quality to this review article for short communication

Ethical approval : Not applicable

Conflict of Interest: The Author declares that there are no conflicts of interest.

Funding : Nil

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The Unseen Positive Effects of Lockdown Due to Covid-19 Pandemic: Air pollution, Sound Pollution, Water Pollution, Sanitation and Hygiene, Behavioral Change, Global Warming, Road Traffic Accidents

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Abstract:

Background: This study states that, the environment became polluted even after consistent efforts by various governments and organizations. Pollution due to various factors was out of control all over the world and it was badly affecting human lives as well as the mother earth. It was very essential to control these pollutants because the environmental and health interaction was closed, but it was not possible practically. Lockdown due to Covid 19 has played a very vital role in controlling these pollutants; it has given a much needed break to our mother earth.

The outbreak of Covid-19 viruses started in Wuhan, China in the month of December 2019. After its outbreak, it’s called as Covid-19 worldwide. By March 2020, World health organization declared it as a global pandemic disease. This disease has forced complete world at almost a stop these days no human movements are allowed unless and until it is really needed or is an emergency situation, the government has taken very strict steps to control the spread of this disease all over the world and various country governments have declared lockdown in their respective countries. Everybody is restricted from moving outside their houses, even all vehicles are restricted, shops and factories are compulsorily restricted from functioning. Closed factories and companies are showing considerable positive effects on health. Otherwise in developing country like India environmental pollution has serious impact on people’s health it was badly affecting human respiratory and cardiac system and due to which peoples were losing their lives. Water pollution was affecting human digestive system, renal system. But now due to controlled global warming communicable diseases are reduced. Ban on vehicles reduced traffic pollution, reduced accident rates too. Peoples are at rest at their houses, they are showing many positive behavioral changes as they are spending quality time with family, they are engaged in cleaning their home regularly. Due to this lockdown unlike human being’s nature too got its much needed break after many centuries. Conclusion: Aim of this study is to compare the situation of pollution and other related factors before and after lockdown along with its comparison and to identify unseen positive effects of the Covid-19 lockdown which we have never seen before. If all these positive effects continued for a long time, then it will definitely become beneficial for the living creatures as well as to mother earth.

Key word: Covid 19, Effects, Lockdown, Positive, Pandemic, Unseen.

Introduction

The world has never experienced a lockdown situation arises due to Covid 19 before, almost all the countries in the world are under influence of Covid 19. In India, to control spread of Covid 19 pandemic disease government has declared complete lockdown since 25th March to 14 April 2020 and it is now extended up
to July 31, 2020 to break chains of Covid 19 through maintaining social distancing.

Covid-19 pandemic lockdown has shown never seen before positive effects all over the world, which was otherwise not possible in the world through human efforts. People all over the world are at home, transportation has completely been stopped and industrial shutdown has been imposed as a precautionary measure to control the spread of Corona virus worldwide. It is showing so many positive effects on factors like air pollution, sound pollution and water pollution, global warming, other communicable diseases, sanitation, hygiene, behavioral changes in human beings too seen, controlled road traffic accidents and many others. These positive effects can be seen after lockdown during covid19 pandemic.

1. Air pollution

In India, the main motto behind imposing nationwide lockdown was to stop the spread of the novel corona virus, as by studies we came to know that this virus spreads through the air, droplets, touch and hence social distancing should be maintained.

Almost all factories, markets, shops, and regional places are closed all over, public transport is also suspended, construction work is halted, the government of India is forcing its citizens to stay home and practice social distancing whenever they go out for unavoidable reasons. So this closure of factories and transport, etc. are showing very good signs with respect to reduction of Air pollution.

Sources of air pollution

There are various sources responsible for air pollution.

1. Vehicles are one of the major causes of air pollution as they release pollutants like Carbon dioxide, Carbon monoxide, Ozone, Sulpheredioxide, Hydrocarbons etc.

2. Industrial wastes are also responsible for air pollution, thermal power stations produces heat which is very harmful for the environment and health.

3. Anthropological sources are responsible for respiratory diseases and cardiovascular diseases.

But after lockdown all sources of air pollutions and pollutants are under control, which helped to reduce respiratory diseases and cardiovascular diseases among vulnerable population in India.

Impact of Air Pollution

Air pollution not only causes severe effect on the environment, but also on human beings too like it damages the lungs in ageing people, it causes allergic irritation in the sense organs. It results in reduction of life span of people. Acid rain destroys soil nutrients. But all these severe effects are somewhat under control due to lockdown as pollutants released into the air are under control.

After lockdown, Air quality in cities like Delhi and Lucknow is improved. The most significant impact was seen in PM2.5, NO2 and CO levels. During the lockdown period the SO2 levels showed less significant decline. Harmful gases like nitrogen dioxide, etc., which are released by vehicles and power plants, etc., are very harmful with respect to the health of the people. These substances are dangerous as it can reach deep into the lungs and can spread into other organs and the bloodstream, can cause serious health issues. Now, due to lockdown air pollution is under control, hence people are feeling more comfortable; their visits to the Doctor’s clinic are decreased up to considerable level.

Research Facts about Air Pollution in India

It is observed that, most of companies do not follow rules with respect to the environment and safeguard the community people. A survey done in 2015 showed that, India is world’s seventh environmentally unsafe country. Bangalore is declared as an asthma city as around 30 % children are suffering from asthma caused due to air pollution. The cancer patient’s rate is also increasing in India. As per the survey done by the National Cancer Control Program by the year 2026 about 1.4 million people may suffer from cancer.

Earlier, due to numerous natural and anthropogenic causes, Northern India was considered as one of the major hot spots of pollution until lockdown in 2019 due to various sources such as dust, industrial and vehicular pollution, etc, this region was always considered as most polluted area. These sources were harming air quality and peoples safety. One of the key sources of pollution
was the dust storm, which occurred every year during the month of March-June\(^4\).

Now a day’s air quality in India’s major cities is improving by 60 % as compared to last year\(^5, 6\).

<table>
<thead>
<tr>
<th>Table: 1 Air quality according to categories</th>
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<tbody>
<tr>
<td>Good air quality</td>
</tr>
<tr>
<td>Satisfactory</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Poor</td>
</tr>
<tr>
<td>Very poor</td>
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<tr>
<td>Severe</td>
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</table>

According to Central pollution control Board’s National air quality Index data analyzed by ET Energyworld, In Delhi during the lockdown air quality is satisfactory as compared to last year\(^7\).

<table>
<thead>
<tr>
<th>Table: 2- Air quality in Delhi Before and after lockdown</th>
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<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>March 25</td>
</tr>
<tr>
<td>March 26</td>
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<tr>
<td>March 27</td>
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<tr>
<td>March 28</td>
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<tr>
<td>March 29</td>
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<tr>
<td>March 30</td>
</tr>
</tbody>
</table>

*Small particulate matter of 2.5 microns or less in diameter.

2. Sound pollution

Sound pollution too makes an adverse impact on human health and their activities. The main sources of sound pollution are vehicles sound, transportation etc.

Before lockdown sound pollution in India was horrible, noise pollution was affecting both physical health and mental health of the people. Higher or continuous sound for a long duration can damage physiological health. Noise pollution is associated with several health conditions, including cardiovascular disorders, hypertension, high stress levels, tinnitus, hearing loss, sleep disturbances etc\(^8\).

But after lockdown from the month of March, traffic is minimized on road, factories are not allowed to function; social functions and large gatherings are banned, so due to this and other such reasons sound pollution has reduced to a considerable level.
Table: 3 Sound pollution in Delhi in the month of April in 2019 and after lockdown sound pollution in 2020 in the same month reduced at level 9.

<table>
<thead>
<tr>
<th>Sound pollution</th>
<th>Delhi in April 2019</th>
<th>Delhi in April 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average 100 dB and higher</td>
<td>Average 40-50 decible</td>
<td></td>
</tr>
</tbody>
</table>

3. Water pollution

Restrictions on the sailing of ships and boats in the water are becoming very useful with respect to cleaning of water resources. This has never been happened earlier and it was never ever possible to clean water resources with such purity by human efforts for even developed countries. These changes are observed these days due to non-interference of humans in all types of waters, a ban on ship transport, cruises and fishing ships, etc. In general we can say nature is having its time. Fishes are easily visible or fearlessly coming out of water in many cities of the world. Many famous rivers in the world are more clear now like the river Ganges in India is very clear from its source due to lockdown, the government is trying its level best to clear it for so many years but never succeeded but lockdown did impossible possible 10.

As most of the major industries are closed, it is resulting in saving of water up to a large extent, as industries use huge amounts of water during various processes like cleaning, washing, etc.

Industries generally flow their harmful waste water into the sea or in a river and due to that drinking water became contaminated and contaminated water caused waterborne diseases among children’s 1. But now a day as industries are closed this waste water is not at all mixing into the regular water sources and this is directly affecting in the clear and pure water.

Diseases, causes due to water pollution in children’s

Diarrhea,

This is a common waterborne disease that often affects children under the age of five. In severe cases; the symptoms include dizziness, fatigue, pale skin, and loss of consciousness 11.

Cholera

Cholera is caused by contamination of food or drinking water by the bacteria Vibrio cholera. The symptoms are like diarrhea, cramps on the abdomen. It occurs mainly in children and causes morbidity and mortality 11.

Typhoid

Typhoid is caused by contamination of food or drinking water by the bacteria salmonella typhi bacteria transmitted by contaminated water. The patients developed prolonged episodes of fever, loss of appetite, nausea, headache, constipation, and loss of body weight 11.

During lockdown the government is playing very essential role in improving access to safe water and sanitation of the areas, a number of steps undertaken at the individual level are also proving very essential in cleaning of water.

4. Sanitation and hygiene

Sanitation and hygiene level has tremendously increased in India during this period to avoid spread of Covid 19.

Study report on challenging to Hygiene Improvement in Developing Countries in 2019 stated that hygiene is a condition or activity that should be preserved in the community in order to avoid communicable diseases and improve social outcomes. Lots of developing countries are struggling to maintain this. Most of these challenges include poverty, lack of political participation, lack of full community participation, inadequate gender equality, lack of effective interaction among community and behavioral problems. Several measures have been proposed to reduce these challenges, including community 12.
During lockdown due to Covid-19 pandemic every hospital is also taking extra efforts to maintain cleanliness all over the world to prevent cross infection and contamination.

So it is observed after lockdown that, more awareness is created between community people and health care personnel towards sanitation and hygiene from various sources and they are implementing it too.

5. Behavioral changes during lockdown

Physical Health: Community peoples are more aware regarding social distancing, hand washing practices, wearing masks, cleaning and reusing masks. They are also aware about steps to be taken after continuous wearing mask if they developed mild headache, nausea vomiting etc.

1. They are paying special attention towards their health like regular exercises, diet control, etc., it helps to control blood pressure, blood sugar, free from stress and tension.

2. People these days are trying to understand their family, their needs which is resulting in a strong bond amongst themselves and it’s a good chance to forget and forgive family affairs through good interpersonal relationship.

3. They are getting close to the nature, many peoples who are at their native places or villages for relaxation and are spending more time with nature by activities like cleaning of the gardens, watering plants, farming etc.

4. They are gaining knowledge as reading habit has been increased due to lockdown, as peoples are now having lots of free time they have now developed the habit of reading, which is resulting them in gaining knowledge and improving memory.

5. People have started saving resources like money, grains and vegetables, etc., they are using them as per actual need only. This lockdown has showed people’s uncertainty of life and has taught them the importance of saving resources that may be money or other resources.

Mental Health: Human beings all over the world are continuously engaged in various activities in a generation; they were over-worked, over-burdened since generations and finally got this much needed rest. While maximum of them are working from home, they are these days not forced to cross polluted traffic to reach the office, they are getting quality time to spend with their families. They got time after so many generations to read, to sleep peacefully and to eat to their heart’s content this will indirectly effect on human health, will help in reducing human stress and in developing human body strength, reducing diseases in current and coming generations.

In a report on the effect of working hours on sleep and mental health in 2017, indicated that longer working hours cause the poor physical and mental health of the individual. Too long working hours are having adverse effects on quality of sleep. It’s a rather significant problem that has impacted both employees and the profitability of the company. The association between these symptoms and the sleep disturbances was positive.

But now after lockdown every person got a break from their busy schedule for self realization.

A moment of self-realization: The great outcome of this Lockdown is we realized the need of making changes in our lifestyle and thought process. Now the condition is that youngsters are getting enough time to share their feelings, ideas and valuable thoughts with their family members and friends and colleagues. It’s a very good time for younger’s to keep stress free mind.

If everybody will keep themselves engaged like this in their lives there will be no pathway to come negative thoughts in a person’s mind. After lockdown in the future if this situation will be continue ultimately a reduction in mental illness and suicidal cases due to stress and tension among younger’s in India may be observed.

5. Road traffic accidents

There are almost no people or less people on the road due to lockdown resulted in less number of accidents and street crimes. Police presence in almost every vital place and closed borders has helped in low rate in burglary and vehicle thefts all around the world. Proper checking at the borders has restricted criminals from entering the borders of other districts or states.

This lockdown has shown a positive impact on road traffic accidents too, apart from its original purpose of controlling the spread of Covid-19 as only essential
vehicles are only allowed on road with permission; it has reduced accident rates too.

Decrease in drunk and drive cases are also remarkable during this period as wine shops all over have been shut down. Accidents due to youngsters rash driving have also decreased these days as they are not allowed to move outside due to strict police and traffic control arrangements by various governments.

No constructions services are allowed to function during this period also factories and industries manufacturing non-essential goods too have been shut down till further notice has created a very positive effect on related resources and decrease in pollution as with respect to different studies industrial pollution accounts for at least half of the pollution in the country, especially in populated cities like Delhi, Mumbai, Kolkata, Bengaluru and so on. Traffic movement has been reduced to almost zero in all the cities around the country. Various photos of deserted roads have flooded on social media, with more and more people staying indoors and not daring to move out of their homes during this outbreak of Covid 19 pandemic.

Mortality and injuries resulted from road traffic accidents are serious issues in India and are increasing general medical, social and economic issues. Approximately 2,650 people die annually and 9,000 get injured every year due to traffic accidents. In 2013, the most recent year that data is available for, 137,423 people died and 469,900 people were injured as a result of road accidents in India. The distribution of road traffic deaths and injuries has been found to be different according to age, month, and periodically. The people of the economically developed age group 30-59 years were the most vulnerable among people of all age groups. It was concluded that India is facing the worsening situation. There is an urgent need to consider the difficult situation of road deaths and injuries.

Table 4: In Acharya Vinoba Bhave Rural Hospital, DMIMS, Sawangi, Wardha, Maharashtra, India. In causality in between March to June 2019 daily 15-16 road accident cases were registered and in 2020 in between March to June daily only 5-6 cases are registered due to road traffic accidents.

<table>
<thead>
<tr>
<th>Road traffic accidents cases</th>
<th>In 2019 in the month of March to June in an average patients are registered</th>
<th>In 2020 in month of March to June in an average, patients are registered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1,830</td>
<td>610</td>
</tr>
</tbody>
</table>

Benefits of reduction in accidents cases

1. Less Hospitalization.
2. Decrease in long term disability among younger generation and others.
3. Decrease loss of life and injury.
4. Decrease in financial burden on family.
5. Reduced stress and tension among family members.

2. Global warming

This lockdown due to Covid-19 is showing a number of positive effects on earth; in fact, we can even say our planet is getting a much-deserved rest for so many years. World temperature was at its high due to global warming, various kinds of pollutions, etc., which was otherwise not possible to reduce or to adjust at a normal level but this lockdown has helped a lot in reducing this high temperature. As cities now can see blue skies and clearer water, water, animals to enjoy this phase as there is no human interference and increased purity level of water. Areal images taken from satellites...
of various countries from space are clearer than ever due to this clean and clear level of air. In coming future this deduction in global warming will definitely result in avoiding thousands of deaths due to global warming as well as it will help reduce some dangerous diseases all over the world\textsuperscript{17}.

Earlier as per study the effects of global warming on South Asia were very thinkable like steady sea level rise, increased cyclonic activity, and changes in ambient temperature and precipitation patterns. Increased landslides and flooding were projected to have an impact upon states such as Assam. Rise in sea level had a submerged several low-lying island in the Sundarbans, displacing thousands of people. The first among the countries to be affected by severe climate change was Bangladesh. There was a reduction in fresh water availability, disturbance of morphological processes and a higher intensity of flooding. Regarding local temperature rises, it was 3.3 °C with the min-max range as 2.7 – 4.7 °C. For Tibet it was 3.8 °C and min-max figures of 2.6 and 6.1 °C \textsuperscript{18}.

In 2019, India witnessed high temperature second – longest period in the last 31 years between March 7 and June 2. Long heat waves swept through 23 states which killed almost 300 people in India\textsuperscript{19}.

Impact of excess heat on the human body

After over heating the human body requires evaporative cooling even in low activity movement. If the heat level exceeds, humidity in human body shows the adverse effect as it can convert into Dehydration, Hyperthermia and Heat stroke. Heat illness effects on many organs and system, including brain, heart, kidney, liver, etc\textsuperscript{20}.

Recent research has mentioned that prolonged exposure to heat causes physical exertion and dehydration; these factors can lead to chronic kidney disease in India \textsuperscript{21}.

But due to lockdown in the year 2020, temperature, level in-between the months of March to June is low as compared to last year 2019. In future also if the earth gets a similar pollution free and heat waves free break, then ultimately heat exposure, illness and diseases will be reduced.

In Mumbai and Delhi temperature is decreased as compared to last year\textsuperscript{22}.

<table>
<thead>
<tr>
<th>Table: 5 Average temperatures in Delhi and Mumbai, Maharashtra, in month of March to June in 2019 and 2020 during lockdown period.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In India climate Temperature</strong></td>
</tr>
<tr>
<td>Average Temperature</td>
</tr>
<tr>
<td>March Max 450C Min 390C</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>March to June 2019 in Mumbai, Maharashtra</td>
</tr>
<tr>
<td>Average Temperature</td>
</tr>
<tr>
<td>March Max 350c Min 320c</td>
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</tbody>
</table>
Overall this Covid 19 lockdown has increased so much positive and good signs in people all over the India, if people continue these good practices, even after Lockdown, then it will definitely help in decreasing communicable diseases in India in coming future.

Discussion

It was not possible ever to control all types of pollution or remove pollutants from air, water, etc. Due to this mandatory lockdown nature got a break after so many centuries where there is no human interference, no pollution and hence automatic nature got its time to clear it all and health positive effects are also seen.

After lockdown if Government takes strict steps to control various types of pollution, the rate of related diseases will ultimately reduce.

If sanitation and hygiene maintenance will continue communicable disease prevalence will reduce in adult and children.

Road traffic accident reduction can prevent long term disability and mortality among people especially in younger’s.

If younger’s will get adequate time for themselves, then ultimately a reduction in mental illness and suicidal cases in younger’s can be maintained.

Conclusion

This compulsory lockdown has shown many positive effects all over the world, in the most important is air pollution has decreased to almost zero, which has shown improvement in the Ozone layer. Global warming has also reduced to a considerable level. People all around the world are more serious about sanitation and hygiene. Lockdown has also shown positive effects on both human animal behaviors, both are in their respective places without any interference and are enjoying their time. Accident rates have increased to a considerable low. Overall, this lockdown has many never seen effects on human life, the environment and animals.

Even after this Covid 19 lockdown, if we succeed in maintaining same sanitation and hygiene, limited use of resources, etc. then it will become definitely beneficial to coming generations.

Acknowledgement

The Author is thankful to Dr. Seema Singh, Professor cum Principal, Smt. Radhikabai Meghe Memorial College of Nursing. Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi (Meghe) Wardha, Maharashtra India. for her timely support and valuable suggestions. The author also thanks Mrs. Jaya Gawai, Dean academic Dept. of mental health Nursing, for her timely supports. Extended gratitude to Dr. Amol Bhawane, Assistant. Professor Nephrology Dept. Acharya Vinoba Bhave Rural Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University) , Sawangi (Meghe), Wardha, Maharashtra India. for their valuable suggestions and guidance. I would like to thanks my family members for their continuous support during the preparation of this article.

The Authors are also grateful to authors / editors / publishers of all those articles, journals and books, news channels from where the literature for this article has been reviewed and discussed. Author is grateful to journal of medico legal update editorial board members and journal of medico legal update team of reviewers who have helped to bring quality to this manuscript.

Ethical approval: This is a review article so, it’s not applicable

Patient Inform consent: Nil.

Financial support : Nil

Conflict of Interest: There are no conflicts of interest.

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A Critical Appraisal of Bioethical Inclusions in Aetcom Module for Competency Based Undergraduate Medical Education as Prescribed by Medical Council of India

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Abstract

Background – Critical appraisal of AETCOM Module with reference to Bioethical inclusions vis-à-vis professional ethics inclusions needs appraisal for purposes of judicious operational mix of two, so that learner is oriented in all-round manner so that optimal results thereto stand generated with at par status in commensuration with “The UNESCO Universal Declaration On Bioethics And Human Rights”.

Methods – Rapid review of literature with reference to AETCOM Module for identification of Bioethical inclusions in it with reference to Bioethical principles included in UNESCO declaration specially those applicable to profession and not propagation or advocacy. Critical appraisal of identified Bioethical inclusions with reference to their conformity with bioethical principles as applicable to profession included in UNESCO declaration and to work out appropriate inclusion of non-included bioethical principles in form of structured competencies and their incorporation in AETCOM module with reference to their placement, learning levels, mode of assessment.

Conclusion – This study deals with mapping and matching of competencies included in AETCOM module with UNESCO universal declaration on human rights and bioethics giving recommendations which can fulfil realised omissions in present AETCOM module thereby leading to its simplification and adoption.

Key words: AETCOM, AETCOM module, UNESCO, Bioethics, Bioethical principles. Post graduate curriculum, medical education.

Introduction

“Bioethics is a term that has arisen from but is broader than issues of ethics in human research. It addresses ethical issues arising from accelerating technological advances, that can potentially threaten human life itself or affect relationships between human beings and their environment. It focuses on protecting the environment and making it safe for the future generations”¹

The term Bioethics was coined in 1926 by Fritz Jahr in article about a ‘bioethical imperative’ regarding the use of animals and plants in ‘scientific research’².

In the year 1970, the American Biochemist Van Rensselaer Potter used the term to correlate the relationship between the Biosphere and the growing population. It was his work which laid the edifice for what has been known as ‘Global Ethics’, a discipline that centres around the linkage between biology, ecology, medicine and human values³, ⁴.

Sargent Shriver, the spouse of Eunice Kenedy Shriver, claimed that he had invented the word

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“bioethics’ in the living room of his home in Bethesda, Maryland in 1970. He stated that he thought of the word after returning from a discussion earlier that evening at Georgetown University, where he discussed with others a possible Kennedy family sponsorship of an institute focused around the “application of moral philosophy to concrete medical dilemmas”.

Realistically speaking the various posers in regard to ethicality in the domain of medicine in the context of life sciences has not only been a subject of one but to relatively new academic ventures in the field of ‘Bioethics’ and ‘health and human rights’. The core essence of the collage that has been formulated emanates from the concept of human dignity entitled to every individual in the form of an accruable human right independent of any or every discrimination of any and every type including those of gender, caste, creed, colour, faith, belief, religion, ethnicity, trade, occupation, calling, vocation, socio-economic status and geographic location.

It is in this backdrop it is evident that the scope of bioethics continues to expand with the advent of scientific inventions with respect to their rapidity and technological innovations emerging thereto in an open ended manner.

According to Andorno et al, there is interlinkage of bioethics with human rights within the Universal Declaration. International documents have all referred to human rights as well as dignity together and therefore, invocation of human rights has been a crux in laying down global bioethics principles as well. Also, it is being rightly expressed that this interlinkage in between the two entities will foster a new era of development and regulations in the current perspectives of international law.

Taking stock of the overall global situation, the academic committee of the Medical Council of India in the process of formulating a detailed draft pertaining to competency based undergraduate medical education invoked a module titled ‘AETCOM Module’ (Attitude, Communication, and Ethics), which was notified in the year 2018 and is incorporated in the ‘Assessment Module for Undergraduate Medical Education’. It has also been availed for the orientation of full time teaching faculty of the various medical schools, in India under the ambit of the Medical Council of India vide the aegis of National Faculty Development Programme, run through various recognized Nodal and Regional Centers by it as a standing mechanism for the same.

The curricular requirement which was in the context of competencies attributable to Indian Medical Graduate in line and tune with the Global Competencies had to have the vital components of

a. Knowledge (Cognitive)
b. Attitude (Affective)
c. Skill (Psychomotor)

The said curriculum includes structured affective domain in the curriculum and the contemplated competencies thereto are invoked in the AETCOM Module depicting therein in terms of their:

a. Initiation
b. Inculcation
c. Consolidation
d. Certification

The said competency based curriculum for undergraduate medical education that has incorporated AETCOM Module as an integrated part of the said structured curriculum has been notified with the prior approval of the Government of India, by the Medical Council of India and has become operational in the country from the current academic year 2019-20 in an onwards manner.

The United Nations Educational, Scientific and Cultural Organisation was established on 4th November, 1946 with its respective headquarters at Paris, France. Since the inception of UNESCO, the main area of its work has been the ethical issues in relation with science and scientific advancements especially, those made in the direction of life sciences along with devising and developing normative standards on an international level. As rightly mentioned by Aldous Huxley, the first Director General of UNESCO, that if science and scientific perspectives are to be making any contribution to the betterment of mankind, it was essential to balance out the scientific applications against values.
It is in this context that a critical appraisal of the AETCOM Module with reference to its Bioethical inclusions vis-à-vis professional ethics inclusions needs to be looked into for the purposes of an judicious operational mix of the two, so that the learner is oriented on the said arena in an around manner so that optimal results thereto stand generated.

**Aim**

To critically analyze the Bioethical inclusions in the AETCOM Module included in the competency based medical education curriculum for the undergraduate medical education prescribed by the Medical Council of India with reference to Bioethical principles incorporated in UNESCO Declaration.

**Objectives**

1. To identify the bioethical inclusions in the AETCOM Module at various phases incorporated there under with reference to their conformity with Bioethical principles in UNESCO Declaration.

2. To critically analyze the bioethical inclusions in the AETCOM Module so identified in regard to their conformity with reference to Bioethical principles applicable to profession as included in UNESCO Declaration.

3. To suggest update or broadening of the ethical inclusions in the AETCOM Module so as to make them commensurate with the Bioethical principles incorporated in UNESCO Declaration.

**Materials and Methods**

**Period of Study**: 1/1/2020 to 30/6/2020 - six months

**Place of Study**: School of Advanced Studies, JawaHarlal Nehru Medical College, Sawangi(Meghe), Wardha

**Design**: Descriptive Study

**IEC Approval**: Ref. no. DMIMS(DU)/IEC/Dec-19/8671 – From Institutional Ethics Committee, Datta Meghe Institute of Medical Sciences, Wardha dated 31st December, 2019. (attached)

**Procedure**: Rapid review of literature with reference to AETCOM Module for the purposes of identification of the Bioethical inclusions in it with reference to Bioethical principles included in UNESCO declaration specially those applicable to profession and not propagation or advocacy.

Further, to critically appraise the conformity of the bioethical inclusions in the AETCOM Module with reference to their conformity with the bioethical principles as applicable to the profession in the UNESCO declaration.

Then, to work out the appropriate mitigation of the AETCOM Module by incorporating the non-included bioethical principles as applicable to profession incorporated in UNESCO Declaration by structuring it in the form of a competency including commensurate with the level of undergraduate medical education.

**Data**: Identifying Bioethical inclusions in the AETCOM Module and their placement in the competency based medical education curriculum for the undergraduate medical education in vogue from the academic session 2019-20 and onwards in the various medical colleges under the ambit of Medical Council of India.

Critical appraisal of the said identified Bioethical inclusions with reference to their conformity with the bioethical principles as applicable to the profession included in UNESCO declaration.

Further, to work out the appropriate inclusion of non included bioethical principles in the form of structured competencies and their incorporation in the AETCOM module with reference to their placement and learning levels including their mode of assessment.

**Collection Tool**: Critical appraisal of the AETCOM Module document and its placement in the competency based medical education curriculum as against the Bioethical principles as applicable to profession in the UNESCO Universal Declaration.

**Analysis**

1. Bioethical inclusions were identified in the AETCOM Module.

2. They were analysed with reference to their conformity with the bioethical principles as applicable
to profession incorporated in UNESCO declaration.

3. They were appraised to decipher any inadequacy thereto, and the non-inclusions were worked out in the form of core competencies and an attempt was made to incorporate these in the existing AETCOM Module by working out their placement, learning level and modes of assessment, as well.

Results and Discussion

This study analysed a total number of 54 competencies, both core as well as non-core as included in the AETCOM module with their respective learning domains, levels as per Miller’s pyramid, the professional year they are intended to be taught and assessed in as against the principles included in the UNESCO Universal Declaration laid down by the UNESCO.

The competencies included in the AETCOM module were core (n=39) and non-core (n=15). All 54 competencies in the AETCOM module reflected either single or multiple principles as per the UNESCO universal declaration of human rights and bioethics. Therefore all 54 competencies in the said module were identified as professional ethical inclusions. With regards to professional bioethics, 49 competencies were excluded except competencies 3,4,5,7 and 53 which were bioethical inclusions in the wake of the respective basic bioethical principles. So, we have 49 competencies under professional ethics and 5 competencies under professional bioethics in the AETCOM module.

Amongst the UNESCO Universal Declaration, principles 3 to 20 were listed as professional bioethical inclusions which were directed towards the learner and his profession directly. The remaining principles were directed towards the states, international affairs and regulation i.e. advocacy. These were therefore excluded.

So, we had two sets – one set of AETCOM module competencies and another set of UNESCO universal declaration principles.

Of the total number of AETCOM module competencies, 21 competencies had their learning level as K standing for Knowledge or the cognitive domain as per Bloom’s Taxonomy and KH level standing for Know How as per the Miller’s Pyramid. Remaining 23 competencies had their learning level as S standing for Show or the psychomotor as well as the affective domains s per Bloom’s Taxonomy and SH level standing for Show How as per the Miller’s Pyramid.

Therefore, it can be noted that 21 competencies dealt with the cognitive domain and 23 competencies dealt with higher domains of learning – psychomotor and affective.

The verbs utilized for the cognitive domain were “enumerate, describe, identify, discuss and defend” respectively. The verbs utilized for the higher domains of learning were “demonstrate, administer, communicate, identify, discuss, defend” respectively. The common verbs utilized in both cognitive as well as higher domains of learning were “identify, discuss and defend”.

Article 18 – decision making and addressing bioethical issues was reflected in maximum number of competencies (n=44 competencies). This was followed by article no. 3 – Human dignity and human rights (n=33 competencies) and Article 4 – Benefit and harm (n= 30 competencies). Further, article 13 – solidarity and cooperation was noted to be reflected in 17 competencies followed by article 8 – respect for human vulnerability and personal integrity (n=10 competencies). Then article 5 – autonomy and individual responsibility was reflected in 9 competencies and articles 6,9 and 10 (article 6 – consent, article 9 – privacy and confidentiality, article 10 – equality, justice and equity) were reflected in 8 competencies each. Article 7 – persons without the capacity to consent was reflected in 7 competencies followed by articles 11 and 12 (article 11 – non-discrimination and non-stigmatization and Article 12 – Respect for cultural diversity and pluralism) were reflected in 6 competencies each. Articles 14,15 and 20 (Article 14 – Social responsibility and health, Article 15 – Sharing of benefits and Article 20 – Risk assessment and management) were reflected in 5 competencies each. This was ultimately followed by article 19 – ethics committees (n= 3 competencies). Thus the article which had the maximum reflection was Article 18 – decision making and addressing bioethical issues(n=44 competencies) followed by article no. 3 – Human dignity and human rights (n=33 competencies) and Article 4 – Benefit and harm (n= 30 competencies). The article 19 – ethics committee had the least reflection
(n=3 competencies).

However, the articles 16 and 17 (article 16 - Protecting future generations and Article 17 – Protection of the environment, the biosphere and biodiversity) had no reflection in the AETCOM module.

These principles need to be articulated in the AETCOM module by working out their placement, learning level and modes of teaching and assessment.

Suggested competencies framed for commensurating article 16 – Protecting future generations –

1. Describe the impact of life sciences on genetic constitution and future generations with regards to medical profession.

2. Identify, discuss, defend and demonstrate the impact of life sciences on genetic constitution and future generations with regards to medical profession.

Suggested competencies framed for commensurating article 17 – Protection of the environment, the biosphere and biodiversity –

1. Describe the interconnection between human beings and other forms of life.

2. Describe the importance of appropriate access and utilization of biological and genetic resources.

3. Describe the importance of respect for traditional knowledge.

4. Describe the importance of role of human beings in the protection of the environment, the biosphere and biodiversity.

5. Identify, discuss, defend and demonstrate the interconnection between human beings and other forms of life.

6. Identify, discuss, defend and demonstrate the importance of appropriate access and utilization of biological and genetic resources.

7. Identify, discuss, defend and demonstrate importance of respect for traditional knowledge.

8. Identify, discuss, defend and demonstrate importance of role of human beings in the protection of the environment, the biosphere and biodiversity.

The competencies framed for article 16 are two – first covering the cognitive domain and second covering the psychomotor and affective domain with K,KH and S,SH levels in Miller’s pyramid respectively. The professional year in which first competency can be included for teaching and assessment is the third professional year of MBBS undergraduate course for covering the cognitive domain whereas the second competency can be included in the fourth professional year of the MBBS undergraduate course for covering the psychomotor as well as affective domains.

The competencies framed for article 17 are eight – first four covering the cognitive domain with K and KH levels in the Miller’s pyramid and the remaining four covering the psychomotor and affective domains with S and SH levels in the Miller’s pyramid respectively. The professional year in which the first four competencies for article 17 be included for the purposes of teaching and assessment is third professional year of MBBS undergraduate medical education for covering the cognitive domain and the remaining four competencies be included in the fourth professional year for the purposes of covering the psychomotor and affective domains.

Modes of teaching these competencies are didactic lectures as well as large and small group discussions, focussed group discussions. Modes of assessment for the cognitive domains can be in the form of short answer questions, long answer questions or multiple choice questions as a part of the respective theory examination.

Modes of assessment of the psychomotor and affective domains can be in the form of viva voce, oral examination, clinical case presentation, standardised patient and simulated scenarios.

Conclusion and Acknowledgement

The present study deals with the mapping and matching of the competencies included in the AETCOM module with the UNESCO universal declaration on human rights and bioethics. This has brought out subsequent omissions which need to be taken care of in order to make the AETCOM module commensurate with...
the UNESCO universal declaration and therefore, make it better and standardised on global and international level. Omissions so envisaged along with points brought out in this study in the form of recommendations have been supplied with suggested competencies in addition with their placement, learning level and modes of teaching and assessment. These recommendations can be envisaged as suggestions which can fulfil the realised omissions in the present AETCOM module set of competencies and thereby lead to its simplification and adoption.

**Institutional Ethics Committee Clearance letter:-**

**Source of Funding** – Self

**Conflict of Interest** - Nil

**References**

Awareness of Physiotherapy among Higher Secondary Students- A Survey

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Abstract

Background: Physiotherapy is a kind of art among various health care professional. It is the rapidly emerging allied health profession in India. It is engaged with promoting health and maximizing potential. Students are the budding generation where the knowledge regarding physiotherapy as a treatment must be developed. This study focused on students in Thanjavur District since the term physiotherapy as health care service is rising gradually nowadays. The main objective of this study is to scrutinize the level of awareness of physiotherapy among the students who are the vitals of the upcoming generation.

Objective: The purpose of the study is to assess the level of awareness of Physiotherapy among Higher Secondary students in Kumbakonam of Thanjavur District. subjects and methods: A structured survey questionnaire depicting the awareness with three components namely Knowledge Attitude and Belief towards physiotherapy is distributed around 200 students of ARR Matriculation Higher Secondary School in Kumbakonam. The responses were collected from the students for the statistical data analysis. result and conclusion: The awareness regarding all the three aspects were significantly less. It is said that there will be in chance of creating the awareness among students unless the professionals themselves include in the repletion and improvement of the field.

Keywords: Physiotherapy, Awareness, Higher secondary, survey questionnaire

Introduction

According to World Confederation of Physical Therapy (WCPT) it is a profession mainly aims at providing health care to people in order to regain and retain the functional ability throughout the life span.1 The ultimate goal of every physiotherapist is to retain one’s functional ability by all means. The physiotherapist mainly concentrates in rehabilitating a patient and making his independent as possible the condition permits. He is not only a therapist but also a companion to the patient and his family in making them understand the current scenario of the patient and a brief counselling is given to their care taker.

Physiotherapy was initially started by the World’s great physicians namely the Hippocrates and Galen. It played an important role during the First World War and at the time of Polio outbreak. The above mentioned events were responsible for the development and enhancement of Physiotherapy during century back.2 In India Physical Therapy emerged in the year 1952 due to Poliomyelitis. Under the support from World Health Organization, the Indian Government and the BrihanMumbai Municipal Corporation (BMC) undertaken the responsibility in opening the first physiotherapy institution in Seth G.S. Medical College and K.E.M. Hospital in the year 1953.3

Physical Therapy have a wide range of scope. It plays a vital role in every stream namely orthopedics, Neurology, Cardio Respiratory, Sports, Gynecology,
Pediatrics etc. Recent years back the term physical therapy usually meant to deal with pain and assumed that only massage is given by a therapist. According to the study done by Anila Paul in the year 2015 the awareness of physiotherapy in general population is less. Usually patients are referred to physiotherapist by a physician. The therapist must gain confidence from the patient so that the treatment goal will be achieved easily, quickly and effectively. Physiotherapist can work in a hospital or set up a clinic by his own and also have a home visit.

Physical therapy is a highly emerging profession nowadays. The need of physiotherapy in each hospital and health center must be increased. Earlier in medical field the people where more aware about Doctors and also Nurses. Even students of science stream were asked to become a Doctors. This concept is to be changed and people in various cities of our county must be more familiar to the term physiotherapy. It is always said that when we try to impart knowledge regarding anything, it must be given at very early stage. Students are the budding generation where we can take an issue to the next level. Unfortunately students of this generation are lacking in deciding their future career. It is certain that every student must start planning about their future at the time of adolescence. Especially students of rural area in Tamil Nadu are not aware of any recent updates in medical field even if they belong to science stream.

Many studies have been done on medical and non-medical profession, anganwadi workers, and college students regarding the awareness and perspective of physiotherapy in the community. Even studies on school students have been done in Sri Lanka, Saudi Arabia even in North India.

We physiotherapy keenly concentrate in inventing new techniques and exercise protocol for various disability. Recognition of a field in the community not only depends upon the advance treatment available in it, it actually deals with how the technique reach nook and corner of the country. This basic concept is an important factor in physiotherapy as an emerging profession mainly in Tamil Nadu.

As per the knowledge there is no study done in Tamil Nadu on higher secondary students regarding the awareness of physiotherapy. Hence objective of my study is to scrutinize the knowledge of physical therapy among school students in a particular school in Tamil Nadu.

**Methodology**

The study design is a non experimental study-observational type. This survey was mainly conducted among 200 higher secondary students of ARR Matriculation Higher Secondary Students of Kumbakonam. The awareness of physiotherapy was determined using a self-structured survey. The students were selected based on their inclusion and exclusion criteria. Students who fall into the following categories were selected namely age 16 to 18, both Boys and Girls, all groups students and only English medium student were selected. Students who belong to Tamil medium and not willing to participate were excluded from the study.

**Procedure**

Students of ARR Matriculation Higher Secondary School in Kumbakonam were selected based on the inclusion and exclusion criteria. The students belonging to 11th and 12th standard were taken. The study was completely explained to the management and permission was obtained to take information from the students. At first approached the class teacher regarding the study and then each groups in the school were approached and explained the study and a questionnaire were given. After the completion the questionnaire it clearly stated that the data obtained will be used only for the study purposes.

A survey questionnaire depicting the awareness of physiotherapy in three components namely knowledge attitude and belief were constructed and initially distributed to the professionals for any correction. A pilot study using this questionnaire was done with a sample size of 10 for any difficulty in understanding. The pilot study showed a reliability of 0.7. After the pilot study satisfaction the questionnaire were distributed to the school students.

The questionnaire consists of four sections namely, section A includes demographic information (like name,
age, gender, group), section B includes questions that deals with the knowledge regarding physiotherapy, section C includes questions that deals with belief towards physiotherapy and section D includes questions that deals with attitude of physiotherapy. The section B, C and D each questions have two option, YES (OR) NO.

The students were given enough time to complete the questionnaire and the data were collected for the statistics. Statistical Package for the Social Science version 23 were used for the data analysis.

**Data Analysis**

**Table 1 : Knowledge Regarding Physiotherapy**

<table>
<thead>
<tr>
<th>QUESTIONNAIRE</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you aware of physiotherapist</td>
<td>124</td>
<td>25</td>
</tr>
<tr>
<td>have you gained knowledge about physiotherapy from friends/Relatives/Doctors</td>
<td>116</td>
<td>84</td>
</tr>
<tr>
<td>Have you gained knowledge about physiotherapy from mass media</td>
<td>75</td>
<td>125</td>
</tr>
<tr>
<td>is there a physiotherapy clinic in your locality</td>
<td>85</td>
<td>115</td>
</tr>
<tr>
<td>Have you ever visited physiotherapy clinic</td>
<td>53</td>
<td>147</td>
</tr>
<tr>
<td>Have you ever had a conversation with a physiotherapist</td>
<td>48</td>
<td>152</td>
</tr>
<tr>
<td>Have you seen any physiotherapy modality</td>
<td>78</td>
<td>122</td>
</tr>
<tr>
<td>Do physiotherapy work in hospital</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>Do they prescribe medicine and injection</td>
<td>53</td>
<td>147</td>
</tr>
<tr>
<td>Do they take X-ray and blood test</td>
<td>82</td>
<td>118</td>
</tr>
<tr>
<td>Do they monitor blood pressure and administer saline bottle</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Do they have a role at the time of surgery and ICU setup</td>
<td>41</td>
<td>159</td>
</tr>
<tr>
<td>Do you think physiotherapy have a role before and after the surgery</td>
<td>94</td>
<td>106</td>
</tr>
<tr>
<td>Do physiotherapy have a role in First Aid</td>
<td>104</td>
<td>96</td>
</tr>
</tbody>
</table>

The table above shows the response of knowledge based questions regarding physiotherapy.

**TABLE 2 BELIEF TOWARDS PHYSIOTHERAPY**

<table>
<thead>
<tr>
<th>QUESTIONNAIRE</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you think physiotherapy can cure condition that is not possible by medicine</td>
<td>64</td>
<td>136</td>
</tr>
<tr>
<td>Is it necessary for a physiotherapist in a village</td>
<td>66</td>
<td>134</td>
</tr>
<tr>
<td>Do you believe in physiotherapy treatment</td>
<td>149</td>
<td>51</td>
</tr>
<tr>
<td>Is that physiotherapy only deals with pain</td>
<td>81</td>
<td>119</td>
</tr>
</tbody>
</table>
The table above depicts the responses obtained for the questions that deals with belief towards physiotherapy.

**Table 3: Physiotherapy Attitude Based Questionnaire**

<table>
<thead>
<tr>
<th>QUESTIONNAIRE</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do they have a role in pain management</td>
<td>126</td>
<td>74</td>
</tr>
<tr>
<td>Do physiotherapy have any age limit</td>
<td>52</td>
<td>148</td>
</tr>
<tr>
<td>Do they have a role in pregnancy and breast feeding</td>
<td>72</td>
<td>128</td>
</tr>
<tr>
<td>Can a cardiac patient advised for physiotherapy</td>
<td>103</td>
<td>97</td>
</tr>
<tr>
<td>Do they have a role in sports</td>
<td>137</td>
<td>63</td>
</tr>
<tr>
<td>Can a stroke patient advised for physiotherapy</td>
<td>108</td>
<td>92</td>
</tr>
<tr>
<td>Do they have a role in fracture management</td>
<td>135</td>
<td>65</td>
</tr>
<tr>
<td>Do they have a role in breathing difficulty</td>
<td>105</td>
<td>95</td>
</tr>
<tr>
<td>Can a physiotherapy regain an individual day to day activity</td>
<td>123</td>
<td>77</td>
</tr>
</tbody>
</table>

**Discussion**

Among 200 samples. There was about 143 males that is 71.5% and female 28.5% (57). According to this study 62% of students were aware of the term physiotherapy that is the were familiar to the term but the concept of this field was not clear. The knowledge gained about physiotherapy was mainly from their friends, relatives. This study showed that the lack of knowledge was due to misunderstanding the role of physiotherapy with other health professional. Since there are limited number of clinic in the particular locality the students were not able to recognize the role of physiotherapy in the society. In spite of the misunderstanding students were able to find that certain task like prescribing medicine, injection, x-ray was not the work of this profession. One of the previous study in conducted in Ishikawa High School Students states that the term physiotherapy was known to many students by mass media. But in this study the student’s attention towards physiotherapy by means of mass media was very low.

Regarding the role in surgery and ICU was not known by the students as they do not have any idea. As they consider intensive care unit is mainly for the critically ill patient is only under the guidance of Doctors. It seems that students have a confusion on physiotherapy participation in first aid. The indulgent of physiotherapy was not able to distinguish by the students so there was a negative feeling on their treatments. Even they don’t know whether modalities are used in the clinic. This population finds that there is no necessary for a physiotherapy set up in a village, but they believe in it. The students strongly agree that physiotherapy only deals with pain management because as per their knowledge the physiotherapist is confused with masseurs.

According to the attitude pf physiotherapy, the students mostly know the value in sports. There are many electives available in this field but the idea about this is not reached to everyone. This is interesting that since one or two hospital in that particular area are present with very few physiotherapist and patient with fracture or stroke are recommended to the physiotherapist. So the students the value of this field in the fracture and stroke management. As nowadays there are lot of scope for physiotherapist in obstetrics and gynecology, even students considers that there is no role of physiotherapy in this field as they did not taught regarding the scope of physiotherapy in various fields.

As there are many new technology available in physiotherapy and new modalities only very few are aware of this. There is an advent of newer techniques
in physiotherapy, which is supposed to be reached nook and corner of small towns and cities. It is a great disappointment that physiotherapy itself has not reached the small town and cities.

It is stated that the source quality and quantity of information concerning the physiotherapy profession for higher secondary students are very less.

One of the previous study that was done on Kanagawa Prefecture, the high school students nearly 95.4% were aware of physiotherapy. Similar study carried out in Saga Prefecture the percentage of students aware of physiotherapy was about 54. However these studies were not considered as they included students taking part in physiotherapy related organizational skills so that the level of awareness was comparatively high. As physiotherapist our aim should not only deal in preventive and curative measure, there must be a broad community based rehabilitation. This can be achieved by creating awareness in schools of small towns by means of camps, seminars and workshop.

This study reveals that there must be a great initiative in developing the knowledge, belief and attitude towards physiotherapy in every school. This helps the students to understand the role of physiotherapy as a profession and also a career wise.

**Conclusion**

This study was designed to determine the knowledge belief and attitude towards physiotherapy by higher secondary students of Kumbakonam in Thanjavur District. The awareness regarding all the three aspects were significantly less. It is said that there will be in chance of creating the awareness among students unless the professionals themselves include in the repletion and improvement of the field.

**Ethical Clearance**- Taken from Institutional Ethical Committee-Srm Institute of Medical Sciences, Kattankulathur, Chennai.

**Source of Funding**- Self/ or Self.

**Conflict of Interest** - NIL.

**References**


Use of Social Web and Perceptions of Professional Behaviours Regarding Social Web Postings among Physiotherapy Students

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Abstract

Background: Social media websites which facilitate exchange of user-generated content has enormous potential in health care which allows Physiotherapists to post educational information for patients also to share ideas as it acts as a great source of information for research and learning. Objective: To survey students enrolled in professional physical therapy programs regarding their use of social web and perceptions of professional behaviors regarding social web postings. Materials and Methods: A non-experimental observational type of study has been done with 100 Physiotherapy students of three different years and were selected according to the selection criteria. The survey instrument was provided to participate in the study addressing students use of social web. Results and Conclusions: Students are unaware about the social media policy and its importance. Students should learn about the privacy concern and professional boundaries. There is a need for social media policies and education regarding the proper use of social media.

Key words: Social media policy, Digital professionalism, privacy concern, Health care providers,

Introduction

Social media is used as the means of communication for general population as it acts as a great platform for patient engagement and creates opportunities for creativity and innovation for Physiotherapy program. The use of social web is more prevalent among all age groups and professions and this have been connected to health care professionals. They are also provided with tools for sharing information on medico legal aspects of health and on promoting health behaviors. Since social web plays a vital role in providing health information to the community Specific attention is required to address the gap between Physiotherapist and patient in health care delivery service.

Although Social media plays abundant role in health care development and patient engagement, on the contrary it could also possess possible legal, ethical and licensing issues due to its inappropriate use. New professionals becoming familiar with the professional health care environment may be particularly vulnerable to this sort of ethical issues. To avoid such circumstances students must be provided with the knowledge social media policy and what is appropriate/ inappropriate to post on social media. Gragnon and Sabus suggested that developing digital professionalism among Physiotherapy students may result in improving the quality of treatment care and patient wellfare.

Physiotherapists are intended to work with patients quite more often than the general physicians. At times there may arise conflicts of interests between patient and the Therapist in providing best treatment due to difference of opinion of patients on treatment techniques. To overcome such scenario Physiotherapists must provide quality treatment respecting the patient’s privacy concerns. Provided with the social media policy still queries remain on how to maintain professional boundaries with patients while using social media.
Studies show that seeking information about patient through online is actually helpful in knowing the truth and also in gathering more information on patient’s condition. This information seeking could get affected by intruding privacy concern of patient and uncleared ethical dilemmas regarding professional boundaries. It also prevents its users from benefitting through social web interaction.

Health care professionals using social web sites must focus on their responsibilities as their postings on the wall could reflect their professionalism. The adoption of Digital professionalism and discussion on social media policy may be the best method to teach students on the appropriate use of social media. However proper data on the physiotherapist students use of social media and its postings is required to implement such discussion forum. Hence this study aimed to survey students regarding their use of social media, social media postings, their judgements about behaviors of others using social media and their own rating of privacy concerns.

**Materials and Methods**

**Participants:** This study surveyed Undergraduate Internship and first and second year Post Graduate Physiotherapy students. The survey instrument was distributed to all the three-year students (N=100) of both the genders who were volunteered to complete the survey. It was limited to particular group based on their clinical exposure and practical experiences of duration of minimum 6 months.

The survey instrument used in this study was similar to the Instrument used in the other published studies enquires about Familiarity with their professional program and its social media policy, personal use of social media. Also enquires about posting habits, rating of behaviors according to their perception, acceptable use of social media and privacy concerns relating to various scenarios. The focus of this study was aimed at the students’ knowledge of social media policy, rating of acceptable behaviors of other people’s posts to social media site and rating of level of privacy concern to various scenarios which is applicable to their clinical practice.

**Data Analysis**

The statistics were calculated using the SPSS 20 software. Descriptive statistics were made on the participants gender, year of program and age. The frequency and percentage of each response under category were calculated and tabulated.

**Results**

**PARTICIPANTS:** This study surveyed Physiotherapy students of about 100 in total. Among which 57 participants are from Undergraduate Internship, 19 from Post Graduate first year and 24 from Under Graduate second year which included 50 male and 50 female participants. When asked about their professional program’s social media policy 74% of the respondents did not know about it. 79% did not know if their sites were being monitored by their professional program but almost 70% of the respondents thought that employers would monitor their social media sites as a screening tool prior to their employment.

**TABLE 1- Demographic characteristic of the participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male participants</td>
<td>50</td>
</tr>
<tr>
<td>Female participants</td>
<td>50</td>
</tr>
<tr>
<td>Year of study</td>
<td></td>
</tr>
<tr>
<td>Internship</td>
<td>57</td>
</tr>
<tr>
<td>PG year1</td>
<td>19</td>
</tr>
<tr>
<td>PG year2</td>
<td>24</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
</tr>
<tr>
<td>20-22</td>
<td>50</td>
</tr>
<tr>
<td>23-25</td>
<td>46</td>
</tr>
<tr>
<td>&gt;26 years</td>
<td>4</td>
</tr>
</tbody>
</table>

**Social Media Policy:**

When investigated about whether their institution had social media policy, 74% of the respondents did not know about it, 82% responded that they did not read about the social media policy in their institution and 79%
of respondents mentioned that their professional program did not monitor students’ social media sites. The numbers 1, 2 … 7 represents the questions asked regarding social media policy.

![Graph 1- Knowledge of social media policy](image)

**Social Media Behaviors:**

<table>
<thead>
<tr>
<th>Social Media Behaviors</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partially or fully nude</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Smoking</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Holding an alcoholic beverage</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>At parties</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>Kissing a person of the same gender</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>Kissing a person of the opposite gender</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>While intoxicated</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>During sexual activity</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>Using obscene gestures</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>With a patient</td>
<td>11</td>
<td>89</td>
</tr>
<tr>
<td>During physical therapy classroom activities</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>During physical therapy laboratory activities</td>
<td>21</td>
<td>79</td>
</tr>
</tbody>
</table>
### TABLE 3- Rating of students according to their beliefs regarding others’ posts to social media sites

<table>
<thead>
<tr>
<th>Issue</th>
<th>Acceptable n(%)</th>
<th>Not acceptable n(%)</th>
<th>Neutral n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Photograph of someone holding an alcoholic beverage</td>
<td>18</td>
<td>51</td>
<td>31</td>
</tr>
<tr>
<td>A Photograph of someone who is intoxicated</td>
<td>11</td>
<td>68</td>
<td>21</td>
</tr>
<tr>
<td>A photograph that is sexually explicit</td>
<td>10</td>
<td>82</td>
<td>8</td>
</tr>
<tr>
<td>A comment containing profanity</td>
<td>6</td>
<td>85</td>
<td>9</td>
</tr>
<tr>
<td>A comment containing discriminatory language</td>
<td>4</td>
<td>91</td>
<td>5</td>
</tr>
<tr>
<td>Disparaging remarks about one’s institution</td>
<td>8</td>
<td>81</td>
<td>11</td>
</tr>
<tr>
<td>Disparaging remarks about faculty</td>
<td>10</td>
<td>79</td>
<td>11</td>
</tr>
<tr>
<td>Disparaging remarks about one’s health profession</td>
<td>7</td>
<td>89</td>
<td>4</td>
</tr>
<tr>
<td>A description of a patient encounter</td>
<td>10</td>
<td>74</td>
<td>16</td>
</tr>
</tbody>
</table>

### TABLE 4- Responses of participants indicating awareness of classmates engaging in negative behaviors

<table>
<thead>
<tr>
<th>Issue</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posting information that violates patient confidentiality</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>Posting examination questions online</td>
<td>19</td>
<td>81</td>
</tr>
<tr>
<td>Ridiculing or making fun of another classmate</td>
<td>38</td>
<td>62</td>
</tr>
<tr>
<td>Ridiculing or making fun of a faculty member</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Posting pictures that you feel reflect unprofessional behaviour</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

### Social Media Usage

### TABLE 5- Rating of students according to their beliefs regarding the acceptable use of social web

<table>
<thead>
<tr>
<th>Issue</th>
<th>Acceptable</th>
<th>Not acceptable</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Look up patients</td>
<td>58</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Communicate with patients</td>
<td>70</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Send a friend request to a current faculty member</td>
<td>54</td>
<td>21</td>
<td>25</td>
</tr>
</tbody>
</table>
Cont... TABLE 5- Rating of students according to their beliefs regarding the acceptable use of social web

<table>
<thead>
<tr>
<th>Activity</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Send a friend request to a former faculty member</td>
<td>53 23 24</td>
</tr>
<tr>
<td>Send a friend request to a current patient</td>
<td>30 51 19</td>
</tr>
<tr>
<td>Send a friend request to a former patient</td>
<td>31 48 21</td>
</tr>
<tr>
<td>Send a friend request to a current student in your class</td>
<td>71 15 14</td>
</tr>
<tr>
<td>Accept a friend request from a current student in your class</td>
<td>76 12 12</td>
</tr>
<tr>
<td>Use a fake identity</td>
<td>6 86 8</td>
</tr>
<tr>
<td>Conduct an internet search on people with whom you work(stalking)</td>
<td>22 46 32</td>
</tr>
<tr>
<td>Conduct an internet search on classmates</td>
<td>32 39 29</td>
</tr>
<tr>
<td>Conduct an internet search on a patient with whom you are working</td>
<td>26 47 27</td>
</tr>
<tr>
<td>Conduct an internet search for a patient with whom you have worked</td>
<td>23 46 31</td>
</tr>
<tr>
<td>Conduct an internet search of faculty in your professional program</td>
<td>37 26 37</td>
</tr>
<tr>
<td>Conduct an internet search of potential future employers</td>
<td>42 25 33</td>
</tr>
<tr>
<td>Conduct an internet search of potential future colleagues</td>
<td>37 27 36</td>
</tr>
</tbody>
</table>

**Level of Privacy Concern:**

Over 30% of Respondents thought that Posting pictures and videos of a de-identified patient is not a privacy concern. 40% don’t know that it is wrong to enquire about his patient’s progression through a third person. 31% reads an email with de-identified information and 19% had no concern about it. 53% thought it is definitely a privacy concern to share a picture of a patient with a pressure sore with his classmates. 38% of respondents thought that it is not a concern to post a picture of himself/herself with the patients.

**Discussion**

Social media acts as a communication tool between the patient and the Therapist for patient engagement.² The unprofessional content of students in their social websites can reduce their own dignity which in turn reduces confidence of patients towards Therapist.⁴

Health care professionals skips communication through online due to the ethical dilemmas arising related to patients’ privacy concern and posting of inappropriate contents in social media.¹⁴ Digital Professionalism is an indeed method to teach Physiotherapy students regarding social media policy and instruct them on appropriate use of social web sites. Adopting such measures might reduce the chances of ethical dilemmas faced especially by the New professionals.³

Although all the Physical therapy programs have social media policy most of the students did not about its existence and did not read the policy even if they are aware about it. This information suggest that the educational
institution must focus on the social media policies and make sure about its awareness among the students. Since implementation of Digital Professionalism is much more important way in reducing the rates of legal and ethical issues the Physiotherapy educators must support its development.3,6

Students are found to have difference of opinion on what to be posted and what not through online. This could be due to the lack of knowledge and awareness of social media policy. Students' response regarding posting of pictures on social web sites shows majority of participants did not have such experiences whereas few others are found to have posting pictures of classroom and lab activities, with a patient, kissing a person of same gender which is completely depicting their personal activities which has not be addressed in the social web sites. Some behaviors in one’s personal life (harassing, abusing) might have legal impacts on their professional life.10

When the subjects were asked to answer according to their beliefs, few answered that it is acceptable to pass on a comment containing profanity or discriminatory language and few felt acceptable for disparaging remarks about faculty. The appropriateness of social media contents posted by the students might not be felt accepted by others.4,8 So if they have a feel that their social media sites has been monitored by their institution it has to be ensured by the students regarding the appropriateness of contents. Adam G. Pizzuti et al., (2020) concluded that studies are needed to analyse the utilization of social media platforms efficiently for better healthcare related education.15

Students are often unaware that their unprofessional behaviors in the social media sites can have an influence on their Professional life particularly when it comes to violating the rules of Indian Association of Physiotherapy and World Confederation for Physical Therapy. When questions were asked regarding privacy concerns, over 20% of the participants responded that it is not a privacy concern to share the pictures or video of a patient in the social web sites. 38% felt not a privacy concern to post the pictures with the permission of his patient. Over 40% did not know that it is not a privacy concern to enquire the progression of a patient and thinks not a privacy to share information regarding his patient to their friends.

Carlos DeLas Heras- Pedrosa et al., (2020) concluded that patients expect hospitals to identify their problems and interact regarding health care conversation as patient engagement is still lacking in this digital world of communication.16 The rapid development of social media has replaced face to face interaction. It enhances communication by creating a comfortable environment for patients in order to interact for treatment purposes.17

Students must be taught with the social media policy and its importance and they should be reminded that their own perceptions of appropriate of social web postings might not be the same as compared with their patients, faculties and employers. Posting pictures of patients without their consent should be prevented. The proper use of social media by Health care professionals must take the following points into consideration- Be informed, Think before your post something in social web sites. Protect your Professionalism and reputation.18 Class Discussion and workshops on Digital professionalism appears to be the best method of understanding implications, positives and negatives of using social websites.3

Conclusion

Students are unaware about the social media policy and its importance. The perception of students regarding social web postings might not be the same as others and this has to be reminded. Students should learn about the privacy concern and professional boundaries. There is a need for social media policies and education regarding the proper use of social media.

Limitations and Recommendations

The number of participants surveyed in this study is limited. Students were included from various three years of programs having differences in their practical experience. Almost half of the participants are from the Internship the practical experience of Interns is comparatively less than the Post graduates. So future studies can be concentrated on the selective year of program.

Conflict of Interest: Nil

Source of Funding: Self Fund

Ethical Clearance: Taken from Institutional Ethical Committee,
SRM Institute of Science and Technology

References


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Dry eye Disease During COVID-19 : Need for Investment Into Research to Develop Solutions?

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Abstract

Purpose: To review the Clinical Trial Registry of India (CTRI) for clinical trials registered for the interventions for the treatment of dry eye disease.

Methods: CTRI was manually searched by using the different key words related to the dry eye. Trials registered after 2009 were included in the analysis. All the parameters like type of interventions, phase of trial, sample size etc of the each trial were extracted in the predesigned proforma. Descriptive statistics was reported in the form of frequency and percentages.

Results: Total 35 trials were registered since 2010. Majority were phase 4 trials (Post Marketing Surveillance). More than half of the registered trials were related to the ayurvedic products. Most frequent allopathic intervention was lubricant and most frequently used comparator was Carboxy Methyl Cellulose. Range of sample size in different trials was 30-300.

Conclusion: A good number of trials related to the ayurvedic drugs are registered for dry eye. There is a need for similar research efforts for allopathic interventions.

Key words: COVID-19, Dry Eye Disease, Research

Introduction

Coronavirus disease (COVID-19) emerged in December 2019 in China and became pandemic situation worldwide within no time. COVID-19 pandemic has affected more than 215 countries across the globe and many of these countries faced a complete lockdown1,2. Various sectors including routine on job work, academic teaching, field work has come to an stand still due rapid and complete shutdown of all activities3. This had led to increase in scrree time and potentially might cascade to outbreak of Dry eye disease.

Dry Eye Disease (DED), as the nomenclature suggests is a condition characterized by dryness of eyes that may lead to tear film instability, hyperosmolarity, chronic inflammation and neurosensory abnormalities ultimately causing damage to ocular surface4. In contrast to earlier belief, the disease is no longer limited to old age population and has started precipitating in...
early life years of an individual (5). Premature occurrence of DED is often associated with compromised quality of life. In India the reported prevalence of DED ranges from 5 to 35% and is often influence by environmental conditions, life style factors and geographic locations (6, 7). However, there may be substantial gap in terms of claimed prevalence of the condition as significant heterogeneity exist between the methods of diagnosis and there may be an underestimation of the disease due to undiagnosed asymptomatic patients (8). This is partly contributed by the complex and unknown pathophysiology and epidemiology of the disease, subjective symptoms and poor correlation between the discomfort experienced by the patients with the objective clinical scales (9). Mores with pandemic of COVID-19, this constantly evolving definition of the disease might become a public health challenge with reference to its classification, its management and relevance to local context.

With this complex nature of the disease and associated clinical burden, periodic update of the treatment strategies are essential. In last decade several newer treatment agents have emerged with promising results globally (10). However, the landscape assessment of the trials registered from developing countries especially India has not been undertaken yet. Clinical trials forms the main source of evidence-based medicine and hence the backbone of clinical practice. With increase in screen time and possible outbreak of DED, present paper aims to provide a recent update on the clinical trials registered from India to address DED during last decade to understand if there is any need to foster the research on DED.

Methods

We accessed the clinical trial registry (http://ctri.nic.in/Clinicaltrials/login.php-last access on February 2020) using the search terms “dry eye” or “ocular surface”. The present communication is limited to trials registered after 2009 and investigating the effect of drugs on DED only. The trials studying effect of surgical or any other intervention were excluded from the review.

Results

A total of thirty-five trials were registered since the year 2010 and were at different stages of development (table 1). Majority (28%) of the trials were at post marketing surveillance phase (phase 4) and Efficacy trials phase 2 (28%). Very few registered trials were in Efficacy (phase 1) and Multi-centric efficacy phase (phase 3). Though the number of registered interventions for treating DED from India were substantial and comparable with global trends, majority of the trials assessed efficacy of Ayurvedic formulations (66%) (Table 2). Twelve trials (34%) were clinical drug trials where the most frequent category was involving lubricants. It was also observed that around 17% registered ayurvedic formulation trials were retrospective in nature. The sample size in individual trial ranged from 30 to 300. Eight of the registered trials have used Carboxy Methyl Cellulose as comparator, while 10 trials had no drug as comparator.

<table>
<thead>
<tr>
<th>Table 1: Overview of the Clinical trials addressing Dry eye disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of registered drug trials during 2010-2019</td>
</tr>
<tr>
<td>Range of sample size</td>
</tr>
<tr>
<td>Phases of trials</td>
</tr>
<tr>
<td>Phase I</td>
</tr>
<tr>
<td>Phase II</td>
</tr>
<tr>
<td>Phase III</td>
</tr>
<tr>
<td>Phase IV</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Retrospectively registered trials</td>
</tr>
<tr>
<td>Synthetic drugs trials</td>
</tr>
<tr>
<td>Ayurvedic drug trials</td>
</tr>
</tbody>
</table>
Discussion

This study was designed with the aim of evaluating different clinical trials registered in Clinical Trials Registry of India (CTRI). Present evidence synthesis clearly indicates that reasonably good amount of clinical studies are initiated by Indian investigators and also registered to address DED. The review suggests an increasing interest of the Ayurvedic preparation for Dry Eye. India is recognised hub for use of ayurvedic medications with apparently broad spectrum of therapeutic potencies, similar registered trails with Ayurvedic formulations is an expected finding (11,12,13). It also suggest that researchers are looking forward to evaluate age old ayurvedic formulations through well designed, rigorous clinical trial methodologies (14,15). With an increasing penetration of mobiles, net and digital innovations, DED needs to be considered as a serious challenge for ophthalmic morbidity (16,17). There is also urgent need for an advocacy effort towards willingness of the policy makers, clinicians and researchers to identify DED as a public health threat. Lastly a careful follow up for disseminations of these trial results culminating into scientific publication should also be advocated to substantiate findings further. Post COVID-19 pandemic and increased screen time as new normal, there is need to foster and invest into research for novel molecules as DED might create a big potential market looking for need of solutions.

Financial support and sponsorship: Nil.

Conflicts of Interest: There are no conflicts of interest.

Ethical Approval: Obtained from Institutional Ethical Committee, DMIMS, Wardha

References

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**Original Article**

**Covid 19 and Ophthalmic Morbidity among College Students Attending Online Teaching**

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**Abstract**

Due to ongoing pandemic, majority of the academic teaching is executed on online platforms, leading to extended screen time. Anecdotal evidence suggests long-term ophthalmic impact of extended screen time. Present study was carried out to document eye related problems amongst adult university/college students undertaking online classes to document the effects of online teaching. An online survey method was undertaken in the month of July, 2020 amongst university/college students of Gujarat using google form. All possible social media platforms were used to collect the data from students of Gujarat. Epi Info and SPSS were used and both descriptive and inferential analysis was performed. A total of 620 students from the various streams participated in the study where around 56.5% were female respondents. The majority of students (88% of participants) had noticed eye problems out of that 39.1% of participants felt that online teaching as a reason. Headache (54.0%) was the most common problem experienced by the participants followed by Pain in eyes (34%) and Watering (26.3%). To conclude online teaching has led to increased eye strain and other eye-related problems in university/college going students and risk factors for severity included sessions attended per day, the distance at device kept, and the enjoyment level. In spite of the high incidence of ophthalmic issues, none of the respondents contacted any ophthalmologist for the same.

**Keywords:** university/college students, online learning, COVID-19

**Introduction**

Coronavirus disease (COVID-19) emerged in December 2019 in china and became pandemic situation worldwide. COVID-19 pandemic has affected more than 215 countries across the globe and many of these countries faced a complete lockdown. Among all the sectors academic sector was among the first few sectors that faced rapid and complete shutdown of all its activities.¹ Due to this complete lockdown universities have been closed and exams were postpones which creates a huge pressure on higher education institutions and students². According to UNESCO, over 320 million students in Indian schools and colleges are currently impacted.³ Hence the academic teaching is executed on online platforms⁴,⁵,⁶.

Besides this collegians and youngsters are known to have a long duration of screen view be it use computers, tablets, mobile phones, or gaming consoles. The lockdown has further compounded the problem with the virtual classes, making it increasingly difficult to reduce the screen time of young eyes. Digital eye strain (DES), also known as computer vision syndrome, involves a range of ocular and visual symptoms and prevalence of it is 50% or more among the computer users.⁷,⁸,⁹ There are evidences that usual longer screen time are related to ocular symptoms reported like eye strain,
irritation, burning sensation, redness, blurred vision and double vision due to rapid change in accommodative mechanism. These symptoms commonly managed by non-pharmacologically management includes correct ergonomic practices, the use of appropriate lighting while using devices, careful positioning of the digital device, screen distance and taking breaks.

Besides online teaching a current situation of lock down and various restrictions has also led to a rapid change in the routine lifestyle of individuals and there is more dependency on TV, mobile and laptop to watch movies/series, attend webinars and video calls. Present study is focused on college / university going students to evaluate the ophthalmic impact of online teaching. The two main objectives of the study are to document the various effects of online teaching and to identify the associated risk factors.

Methods

An online survey was conducted in the month of July,2020 amongst university/ college going students of Gujarat to document the effects of online teaching. For the data collection google form was created and sent to all students of various academic colleges of Gujarat. All possible social media platforms like WhatsApp, Facebook and emails were used to reach out the maximum number of students. Snow ball sampling method was used to get the more participants. Any university/college going student (irrespective of stream) and wishes to be a part of a survey irrespective of gender and grades/semester can participate. Those who were not willing to participate and not filled the form were excluded from the study and those who filled the forms were considered to have an implied consent to the study.

Data was entered and cross-checked using Excel spread sheet. Data analysis was performed using statistical software like Epi Info version 6.01 and SPSS 20.0. Both descriptive and inferential analysis was carried out to get the better understanding. Inferential analysis was carried out at 95% CI level.

Results

A total of 620 individuals had participated in the study. Out of these total 620 participants maximum participants (69.6%) were from medical stream. Out of 620 participants,56.5% participants were female and majority of them (90%) were belongs to age group 25 years and younger than that. At the time of survey 56.1% participants had glasses. The mean age of the participating students was 21.5 years with a SD of ± 2.9 years. [Table 1.]

About 73% of the total participants attended at least one online teaching session per day and very few participants (5.8%) had attended more than 3 session per day. Each of the online session was around 45-60 minutes. It was documented that only 15.5% participants were enjoying the online teaching over the actual learning while others either did not find any difference between both formats or were not enjoying online session. Majority of the participants (67.3%) used mobile for the online lectures. Almost one fourth of the total participants were not able to attend online classes for more than 30 minutes continuously and felt like leaving in between due to various reasons most common being distraction or pain in eye/watering. On inquiring about distance, they maintain between eyes and device around 46.6 % were not adhering to any distance, whereas around 34% used the device to less than 40 cms. [Table 2.] Apart from online education almost 60% had to undertake 1-3 hrs of additional studying per day. Overall 8.7% of the participants felt they had improved learning by Online teaching.

Table 3. Suggests that more than three fourth of the total participants felt that online learning is more strainful to their eyes. Almost 88% participants had noticed any problem related to eyes. Out of those who included in the present study around 88 % complained eye issues. Out of those 88% (n=545) who had noticed any ophthalmic challenges, 39.1% participants felt that mentioned problems started after beginning of online teaching, however, very few participants (14.1%) had visited eye specialist for the problem experienced. Headache (54.0%) was the most common problem experienced by the participants followed by Pain in eyes (34%), Excessive rubbing of eyes (29.5%), Watering (26.3%) and redness in eyes (15.5%). [Table 4.]

Results revealed that the problems like excessive rubbing of eyes, pain in eyes, redness, headache and
watering were noticed less in the participants who were enjoyed the online classes compared to others. It has been also found that this difference is statistically significant for the excessive rubbing of eyes (p = 0.006), headache (p = 0.007) and watering (p = 0.024).

Above mentioned problems (except redness and watering) were noticed more in the participants who look at the screen at < 40 cms distance followed by > 40 cms distance and variable distance. This difference is statistically significant for the excessive rubbing of eyes (p = 0.007) and pain in eyes (p = 0.002). Those who attended 1 or less than 1 session of online teaching or webinar per day had experienced less problems compared to those who attended > 1 to 3 sessions or > 3 sessions per day. Those who attended more 3 sessions per day experienced more problems related to eyes. Found difference is statistically significant for the pain in eyes (p = 0.000), redness (p = 0.036) and headache (p = 0.033). [Table 5.]

Table 1. Distribution of study participants as per selected socio demographic profile and stream of education

<table>
<thead>
<tr>
<th>Variables</th>
<th>Course Type</th>
<th>Total (N=620)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Engineer (N=72)</td>
<td>Medical (N=432)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14 (19.4)</td>
<td>259 (60)</td>
</tr>
<tr>
<td>Male</td>
<td>58 (80.6)</td>
<td>173 (40)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 20 years</td>
<td>49 (68.1)</td>
<td>187 (43.3)</td>
</tr>
<tr>
<td>21-25 years</td>
<td>23 (31.9)</td>
<td>208 (48.1)</td>
</tr>
<tr>
<td>26-30 years</td>
<td>0 (0)</td>
<td>32 (7.4)</td>
</tr>
<tr>
<td>&gt; 30 years</td>
<td>0 (0)</td>
<td>5 (1.2)</td>
</tr>
<tr>
<td>Glasses</td>
<td>27 (37.5)</td>
<td>258 (59.7)</td>
</tr>
<tr>
<td>Variables</td>
<td>Engineer (N=72)</td>
<td>Medical (N=432)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Course Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 sessions per day</td>
<td>34 (47.2)</td>
<td>324 (75.0)</td>
</tr>
<tr>
<td>&gt;1-3 sessions per day</td>
<td>28 (38.9)</td>
<td>85 (19.7)</td>
</tr>
<tr>
<td>&gt;3 sessions per day</td>
<td>10 (13.9)</td>
<td>23 (5.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you enjoy online learning over actual learning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Device used for online lectures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile</td>
</tr>
<tr>
<td>Laptop</td>
</tr>
<tr>
<td>Tablet</td>
</tr>
<tr>
<td>Either using both or none</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Upto what duration you can continuously attend online class</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 hours</td>
</tr>
<tr>
<td>Upto 2 hour</td>
</tr>
<tr>
<td>Upto 1 hour</td>
</tr>
<tr>
<td>Upto 30 minutes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>At what distance you look at the screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 cms</td>
</tr>
<tr>
<td>&gt;40 cms</td>
</tr>
<tr>
<td>Variable</td>
</tr>
</tbody>
</table>
### Table 3. Effect of online teaching

<table>
<thead>
<tr>
<th>Variables</th>
<th>Course Type</th>
<th>Total (N=620)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you feel online learning is more strainful to your eyes</td>
<td>Engineer (N=72)</td>
<td>47 (65.3)</td>
</tr>
<tr>
<td></td>
<td>Medical (N=432)</td>
<td>327 (75.7)</td>
</tr>
<tr>
<td></td>
<td>Paramedical (N=16)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td></td>
<td>Public Health (N=39)</td>
<td>32 (82.1)</td>
</tr>
<tr>
<td></td>
<td>Other (N=61)</td>
<td>57 (93.4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>473 (76.3)</td>
</tr>
<tr>
<td>Have you noticed any eye problem</td>
<td>Engineer (N=66)</td>
<td>66 (91.7)</td>
</tr>
<tr>
<td></td>
<td>Medical (N=375)</td>
<td>375 (86.8)</td>
</tr>
<tr>
<td></td>
<td>Paramedical (N=14)</td>
<td>14 (87.5)</td>
</tr>
<tr>
<td></td>
<td>Public Health (N=34)</td>
<td>34 (87.2)</td>
</tr>
<tr>
<td></td>
<td>Other (N=56)</td>
<td>56 (91.8)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>545 (87.9)</td>
</tr>
</tbody>
</table>

### Table 4. Descriptive analysis of those who noticed any eye problem (N=545)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Course Type</th>
<th>Total (N=545)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feel that above mentioned problems started after beginning of online study</td>
<td>Engineer (N=66)</td>
<td>24 (36.4)</td>
</tr>
<tr>
<td></td>
<td>Medical (N=375)</td>
<td>141 (37.6)</td>
</tr>
<tr>
<td></td>
<td>Paramedical (N=14)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td></td>
<td>Public Health (N=34)</td>
<td>12 (35.3)</td>
</tr>
<tr>
<td></td>
<td>Other (N=56)</td>
<td>31 (55.4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>213 (39.1)</td>
</tr>
<tr>
<td>Visited eye specialist for these</td>
<td>Engineer (N=66)</td>
<td>11 (16.7)</td>
</tr>
<tr>
<td></td>
<td>Medical (N=375)</td>
<td>46 (12.3)</td>
</tr>
<tr>
<td></td>
<td>Paramedical (N=14)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Public Health (N=34)</td>
<td>7 (20.6)</td>
</tr>
<tr>
<td></td>
<td>Other (N=56)</td>
<td>13 (23.2)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>77 (14.1)</td>
</tr>
<tr>
<td>Use any eye drop for this</td>
<td>Engineer (N=66)</td>
<td>12 (18.2)</td>
</tr>
<tr>
<td></td>
<td>Medical (N=375)</td>
<td>65 (17.3)</td>
</tr>
<tr>
<td></td>
<td>Paramedical (N=14)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td></td>
<td>Public Health (N=34)</td>
<td>9 (26.5)</td>
</tr>
<tr>
<td></td>
<td>Other (N=56)</td>
<td>21 (37.5)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>108 (19.8)</td>
</tr>
</tbody>
</table>

### Table 5. Inferential association with Ophthalmic involvement and other variables and its association with other variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Problems experienced</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

### Table 5. Inferential association with Ophthalmic involvement and other variables and its association with other variables

<table>
<thead>
<tr>
<th></th>
<th>Excessive Rubbing of eyes</th>
<th>Pain in eyes</th>
<th>Redness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enjoying online classes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Much</td>
<td>17 (17.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No difference or not much</td>
<td>166 (31.7)</td>
<td>79 (82.3)</td>
<td>66 (14.5)</td>
</tr>
<tr>
<td><strong>At what distance you look at the screen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 cms</td>
<td>76 (36.2)</td>
<td>134 (63.8)</td>
<td>388 (85.5)</td>
</tr>
<tr>
<td>&gt;40 cms</td>
<td>39 (32.2)</td>
<td>82 (67.8)</td>
<td>19 (14.6)</td>
</tr>
<tr>
<td>Variable</td>
<td>68 (23.5)</td>
<td>221 (76.5)</td>
<td>11 (30.6)</td>
</tr>
</tbody>
</table>

---

**No.of webinars attended**

<table>
<thead>
<tr>
<th></th>
<th>Excessive Rubbing of eyes</th>
<th>Pain in eyes</th>
<th>Redness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 sessions per day</td>
<td>134 (29.5)</td>
<td>320 (70.5)</td>
<td>66 (14.5)</td>
</tr>
<tr>
<td>&gt;1- 3 sessions per day</td>
<td>60 (46.2)</td>
<td>70 (53.8)</td>
<td>19 (14.6)</td>
</tr>
<tr>
<td>&gt;3 sessions per day</td>
<td>17 (47.2)</td>
<td>19 (52.8)</td>
<td>11 (30.6)</td>
</tr>
</tbody>
</table>

---

**Redness**

### Table 5. Inferential association with Ophthalmic involvement and other variables and its association with other variables

<table>
<thead>
<tr>
<th></th>
<th>Excessive Rubbing of eyes</th>
<th>Pain in eyes</th>
<th>Redness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At what distance you look at the screen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 cms</td>
<td>89 (42.4)</td>
<td>121 (57.6)</td>
<td>388 (85.5)</td>
</tr>
<tr>
<td>&gt;40 cms</td>
<td>43 (35.5)</td>
<td>78 (64.5)</td>
<td>111 (85.4)</td>
</tr>
<tr>
<td>Variable</td>
<td>79 (27.3)</td>
<td>210 (72.7)</td>
<td>25 (69.4)</td>
</tr>
</tbody>
</table>

---

**No.of webinars attended**

<table>
<thead>
<tr>
<th></th>
<th>Excessive Rubbing of eyes</th>
<th>Pain in eyes</th>
<th>Redness</th>
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<tr>
<td>0-1 sessions per day</td>
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<td>320 (70.5)</td>
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</tr>
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<td>70 (53.8)</td>
<td>19 (14.6)</td>
</tr>
<tr>
<td>&gt;3 sessions per day</td>
<td>17 (47.2)</td>
<td>19 (52.8)</td>
<td>11 (30.6)</td>
</tr>
</tbody>
</table>

---

**Redness**

<table>
<thead>
<tr>
<th></th>
<th>Excessive Rubbing of eyes</th>
<th>Pain in eyes</th>
<th>Redness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.of webinars attended</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 sessions per day</td>
<td>66 (14.5)</td>
<td>388 (85.5)</td>
<td>66 (14.5)</td>
</tr>
<tr>
<td>&gt;1- 3 sessions per day</td>
<td>19 (14.6)</td>
<td>111 (85.4)</td>
<td>19 (14.6)</td>
</tr>
<tr>
<td>&gt;3 sessions per day</td>
<td>11 (30.6)</td>
<td>25 (69.4)</td>
<td>11 (30.6)</td>
</tr>
</tbody>
</table>
Cont... Table 5. Inferential association with Ophthalmic involvement and other variables and its association with other variables

<table>
<thead>
<tr>
<th>Enjoying online classes</th>
<th>Headache</th>
<th>Very Much</th>
<th>40 (41.7)</th>
<th>56 (58.3)</th>
<th>96</th>
<th>0.007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No difference or not much</td>
<td>296 (56.5)</td>
<td>228 (43.5)</td>
<td>524</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of webinars attended</td>
<td>0-1 sessions per day</td>
<td>232 (51.1)</td>
<td>222 (48.9)</td>
<td>454</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1-3 sessions per day</td>
<td>80 (61.5)</td>
<td>50 (38.5)</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;3 sessions per day</td>
<td>24 (66.7)</td>
<td>12 (33.3)</td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Watering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enjoying online classes</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The current study sought to document the effect of online teaching and to identify associated risk factors. Findings of the study signify that most of the student (88%) experienced eye problem due to online teaching which is similar to prevalence of computer vision syndrome found in engineer and medical students of Chennai. Even there is more free time due to lockdown, students cannot really focus into their study because of this ongoing pandemic effect and their study has affected much more than usual.

Results shows that the only 15.5% students enjoying the online teaching. However, it was found that the almost 74% students liked studying through online teaching in the study conducted by Shatakshi Lall, & Nardev Singh.

Attending classes online without break is strainful to their eyes said to be causing problems like dry eye syndrome, burning sensation in eyes, watering, redness, headache, excessive rubbing of eyes. Out of all these reported symptoms head ache was the most common (50.4%) problem experienced by the students which is quite similar to the study conducted on university students in UAE. This study reveals that those who are attending more than 3 sessions per day experiencing more ophthalmic problems. Joowon Kim et al. also found that longer daily smartphone use was associated with a higher likelihood of experiencing or having ocular symptoms. Advantages of online and Digital education were highlighted by Allen and Saxena.

**Conclusion**

Online teaching has affected the young population which has reported a trend of ophthalmic complaints. Online teaching has increased eye strain and other eye related problems in university/college going students and affected their study. Eye related problems experienced by the students during the process of online teaching are associated with the variables like no. of sessions attended.
per day, distance at device kept and the enjoyment level. Excessive rubbing of eyes is associated with level of enjoyment and at what distance device is kept, pain in eyes is associated with at what distance device is kept and no. of sessions attended, redness is associated with no. of sessions attended, headache is associated with level of enjoyment and no. of sessions attended while watering is associated with enjoyment level.

**Conflict of Interest:** None

**Ethical Approval:** Institutional Ethical Committee, DMIM'S, Wardha

**Funding:** Self

**References**


Lata Kanyal Butola
Tutor, Dept. Of Biochemistry, Jawaharlal Nehru Medical College, Sawangi Wardha, Dmims Maharashtra

Abstract
The dissemination of COVID-19 has contributed to the closing of educational institutions around the world. Such closing accelerated the creation of online learning environments within these institutions, ensuring that learning would not be interrupted. The coronavirus pandemic tested the ability of the centers to cope with a problem that involves online and remote intervention. Most have not been educated, so it is necessary to investigate the motives for providing online classes to students, which go beyond the confinement era. The vast majority of children have no previous experience of remote learning previous to the pandemic. Since the COVID-19 pandemic has destroyed people’s usual lifestyles around the globe, the virtual world has come to the rescue. Like several institutions, schools have already transferred their focus to interactive networks to take classes online. Video conferencing tools such as Zoom and WebEx are commonly used and learning management systems such as Infrastructure Canvas, Blackboard and Google Classroom. In addition, applications such as Proctorio, a Google Chrome plugin that tracks students taking online tests, are available. In India, too, all educational institutions have been suspended by the government as part of the national lockdown, which has impacted learners ranging from school-learning to post-graduate students.

Keywords: E-learning, Lockdown, digital platform, COVID-19.

Introduction

Due to COVID-19 pandemic, the doors of schools and colleges are barred all over the country. Approximately 1.5 billion student studies are affected by COVID 19. The entire education system has changed since the pandemic. The teaching and learning process is part of the digital platforms. This digital platform is known as e-learning. E-learning is a completely different learning experience for Indian students. Web-based computing, distributed learning, online learning, or internet-based learning is another term for e-learning. A method of learning based on formalized learning with the aid of electronic tools is known as e-learning. While education may be centered in or out of schools, the use of computers and the Internet is a core component of e-learning. E-learning may also be defined as a network facilitating the sharing of skills and expertise and delivering education to a wide number of recipients at the same or different times. Earlier, it was not widely embraced as it was believed that this method lacked the human aspect needed for learning. E-learning or online education allows users to obtain access to world-class learning experience where regular higher education may not be feasible due to income, personal or other restrictions. In countries like India, there is a tremendous desire for e-learning to take its shape. India is the second-most populous nation in the world. The power of every nation is its inhabitants, and India has a population of 134 crores, or 1.34 billion. One of the best places in the world to take advantage of these new e-learning developments is a nation like India. Today, India is host to many of the new e-learning developments in education that developing countries have been using for a very long time. As we remember, the whole world is facing a very deadly virus called COVID-19. Due to the worldwide outbreak of Coronavirus, the governments of most countries have directed the closure and advised people to stay in their
homes and be healthy. Likewise, the Indian Government has ordered a full lockdown. Education is known to be the foundation of our nation in India. Owing to the lockdown, the education system has been significantly disrupted because schools and universities cannot be accessed, so the main challenge is how to educate children when living at home. In order to address this issue, the idea of e-learning is being adapted by schools and colleges to educate their students. In fact, e-learning proved to be helpful during the COVID-19 crisis. More students have opted for Ed-tech and other online training outlets during this current pandemic. Online networks such as Vedantu, Unacademy and Byju’s have provided free access to live classes to help students learn easily from their homes, and there has been a huge increase in students using these educational applications. Online platform educators have already begun to take more live classes. Formerly, there were two common categories of e-learning: distance learning and computer-assisted teaching. Where distance learning is preferred for those living in rural areas, information technology is used to give guidance to learners and computer-assisted training uses computer tools to help provide stand-alone digital learning and teaching programs.

**Role in Medical Education**

The need for the hour is to bring e-learning into medical education. The Indian Medical Council (MCI), the governing body for medical education in India, has recognized the value of technology and has included the use of electronic means in the broad competency “Lifelong Learning Dedicated to Continuous Development in Skills and Knowledge”. Indian medical graduates must have acquired this expertise at the time of graduation. The student must constantly learn new skills to keep up to date with the newest technologies, which are generally deemed impossible to do in the absence of technology. The use of e-learning will help them accomplish the aim of continuous professional growth, given the vastness of the program, the shortness of time and the often overburdened schedule. Recognizing the value of information technology for today’s doctors, the General Medical Council in the United Kingdom further claims that medical graduates should be able “to make efficient use of computers and other information technologies, including information storage and retrieval”. From a mere ‘content spreader,’ teachers are gradually becoming ‘learning facilitators’, which is supported by e-learning tools that offer a range of online resources. E-learning systems can be used to increase all learning fields, including cognitive, psychomotor and affective learning. In the cognitive area, classroom instruction may be improved by offering online resources such as pre-reading assignments and audio-video clips during sessions. And students may be equipped with virtual resources such as audio-video clips, podcasts, videos, and web-links for self-directed learning designed to be used at home or as part of Flipped lectures. Psychomotor abilities, while better learned with actual experience, can also be improved by technology, at least up to the ‘know-how’ stage. Audio-visual illustration of treatments, diagnostics and therapies can be given. Students can read through the descriptions and display the procedural skills checklists before actually practicing the same under supervision in clinical posts or in specialist laboratories. In the affective domain, videos of scenarios depicting good and bad communication-skills, role-playing and counselling sessions, and self-recording - can be used to promote learning. Other modalities include multimedia case reports and patient records, health decision support services, interactive patients, medical video games, e-books, e-atlases, instructional libraries, and digital editions of online journals.

More and more colleges are now incorporating classroom training with online learning. Related methods have been applied: Blended learning ties together with online education and traditional classroom teaching. Both blended learning and e-learning in medical schools have developed over the past few years, taking various forms. It should be assured that students have gained a minimum of expertise before they reach the professional. This will significantly increase the efficiency of teaching.

Flipped classroom instruction is a situation where students research challenging data at home. The classroom is then used for problem-solving, analytical instruction and asking and response sessions. This teaching method has been seen to be very effective. Just in time teaching is another type of blended learning, where students study online, respond to MCQs and thus allow the educator to adapt the in-person teaching session to the needs of specific students.
Technology and applications play a vital role in the education sector during the lockdown:

1. Interaction enhancements:

Learning apps can improve a child’s relationship with their guardians. They seem to get more involved and to speak about their professions.

2. Good contact between parent and teacher:

These days, learning innovations are being introduced in different classrooms. This device also allows teachers to lecture from their homes and also review the student’s success with their parents. Teachers will take care of the parents’ questions at any time and wherever and make educated decisions about the child’s development.

3. Availability of resources online:

Online learning infrastructure allows students to browse a broad library of materials, including books, PDFs, test papers and more. These tools assist students in their learning process in order to access informative information.

4. Provides fun too:

According to research, smartphone applications facilitate entertainment. Learning is no longer passive practice, it is involved with apps. Lessons turned into games will change the face of education. The implementation of gamification of learning has helped to increase the level of engagement of children with creative and exciting strategies to play while studying.

5. Promotes distance learning:

Online learning has benefited from versatility in the time and place of study. Students have access to research materials and tools at all times. It also offers students the autonomy to study at their own pace.

6. Offers personalization:

In addition to so many other opportunities, E-learning often provides personalization. In order to address the needs of each student in compliance with their needs, e-learning platforms offer a customized way of teaching and learning that is specific to the individual. They stay involved in the course until they know what to study.

7. Cost-effectiveness:

E-learning technology is much more cost-effective than most other methods of education. With access to all students around the world, regardless of their educational and economic context, e-learning resources are accessible at a low price and provide them with better education.

8. Portability:

When studying online learning applications, you can study in a cozy and relaxing environment. And sure you’re checking it because you’re getting a decent internet connexion.

9. Sustainability:

The method of mobile learning is sustainable. Although millions of trees are being cut down to generate papers on the conventional learning process, smartphone applications in education need only a download. It means greener land for future generations.

10. Report on real-time performance:

E-learning helps students to assess their success in real-time. This input offers students a quicker and more thorough review of their success in school.

11. Live-streaming seminars on the solution of real-time doubts:

Online learning applications allow students to take part in live streaming lectures by instructors and professionals. Students may also benefit from real-time doubt-solving through the use of specialized technologies. This gives them a living knowledge of great and quality education at the comfort of their homes.

E learning during Covid 19

In India, students have access to their studies through e-learning due to a lockout. There are several platforms in place to facilitate online education in India. These networks are funded by the Ministry of Human Resources Development (MHRD), the National Council for Educational Research and Training (NCERT) and the Department of Technical Education. Many apps
have free access for online courses such as Gradeup, Unacademy, Vedantu, Byju’s. In the current situation, students have access to lessons across multiple applications and this lets students study for and complete their exams. Commonly, in India, since closing, most universities and colleges have requested faculty members and students to use the digital platform for academic purposes. Faculty members include PDF files, PPT, tasks, mock-tests to their WhatsApp and addresses, as well as online classrooms through Google Classroom, Zoom Cloud Meeting Software, WebEx App. This study of the students is not affected during this corona crisis. Health educators now face distinct obstacles than their predecessors in training physicians tomorrow. Over the last few decades, improvements in health care implementation and innovations in science have raised pressures on the college staff, resulting in less time for teaching than was historically the case. Changes in health care delivery environments, from acute care hospitals to community-based chronic care facilities, have prompted modifications in educational settings. Seeking time to educate “modern” subjects such as genomics, palliative care, geriatrics, and alternative medicine is challenging while medical school curricula are also being forced to include standard materials. Typical teacher-centered education gives way to a learner-centered paradigm that places learners in control of their own learning.

Benefits of e-learning

- It’s simply a web-based instruction.
- The easiest and most practical way to practice on your own.
- It’s the quickest way to read.
- 24x7 Easy to reach anytime.
- It helps to improve students’ skills when they have the option of choosing study content.
- It’s time-saving. Making education interactive Visualy impressive content to learn and retain better Convenient learning from comfort zones Personalized guidance and mentoring Multiple practice sessions Flexible study time.
- Convenience for participants in the learning process, – streamlining and maximizing the work of teachers.

- Simple, fast and reliable access to tools and deliverables for learning.
- Better data protection.
- More communication opportunities.
- More effective flow of knowledge.
- A highly efficient class organization.

- Some of the advantages of this digital platform are Screen Sharing Online Study-Whiteboard Online Mock Testing and Practice Testing File Sharing Registered and Live Classes Update and share analysis materials detailed scorecards and reviews Annotation Live discussions.

Limitations to e-learning

- It is not effective in rural regions where Internet access is not as high as it is focused solely on the Internet.
- It is not suitable for technical subjects such as mathematics and operational research.
- It is not important that the teacher is always available on request.
- It is not helpful for students who are not self-motivated to learn.
- The use of e-learning can make students feel alienated.

Conclusion

The Covid-19 outbreak makes us realize that no matter where the learning process takes place, schools should be ready to make the most of it. From this review paper we can conclude that e-learning is all about learning and taking a step that helps in moving toward higher education level. We have to be familiar with technology devices and use them to enhance the education system. This tool is very helpful in this pandemic as you will get knowledge without going outside to the home.

Ethical Clearance- Nil

Source of Funding- Nil
Conflict of Interest - Nil

References


Herd Immunity: A Major Advantage or A Major Challenge to Achieve for the COVID-19 Pandemic

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Abstract

COVID-19 caused by the novel SARS-COV2 has been causing worldwide panic in recent times. Global epidemiological findings have shown numerous new cases being reported daily as well as drastic mortality rates. It is the need of the hour to plan out strategies to cope with this ongoing pandemic. Herd immunity has been used as an effective approach for years during epidemics and can prove to be a major advantage in controlling the spread of the disease. Various models have been studied using mathematical estimates to find out how herd immunity can be achieved as well as to predict risks involved in doing so. The current scenario where there are no efficient vaccines available has also been taken into consideration. It is important to take all these facts into consideration to come to a valid conclusion of whether achieving herd immunity can be beneficial or impractical.

Key Words: COVID-19, SARS-COV2, herd immunity, vaccination, pandemic

Introduction

The recently emerged COVID-19 or the so called coronavirus disease is caused by Severe Acute Respiratory Syndrome- Coronavirus 2 (SARS-COV2) and is a deadly disease of recent times that is of major health concern throughout the world. Globally, as of September 2020, there have been approximately 31,370,000 cases that are confirmed of COVID-19, including 966,000 deaths, reported to WHO. The rising number of new cases and the level of mortality across nations makes it a major concern to find suitable means to flatten the curve of the disease and reduce mortality significantly. Various methods to deal with this pandemic are under development, including finding new treatment measures to improve symptoms, isolation of infected individuals to prevent spread, promoting disinfection measures, promoting social distancing and vigorous research to create an effective vaccine.

“Herd immunity or community immunity is a type of immunity that occurs when a portion of the population is immune to a certain disease and provides protection to non-immune individuals in that population.” Herd immunity can be achieved by vaccination or by natural immunity being generated after being infected by the pathogen. It can play a significant role in preventing further spread of the disease in the society, which has also been achieved successfully in the past decades, especially during epidemics. But it is important to understand that achieving herd immunity without an effective vaccine against SARS-COV2 is a challenging task. Moreover, anticipating for target herd immunity to be achieved in a population naturally involves number of people being infected by the disease and may lead to unethical fatality rates. Hence, it is important to understand how herd immunity works to understand whether it is effective to aim at achieving target herd immunity threshold or if the idea itself has too many adverse effects to achieve in real life situations.

Material and Methods

Over the span of a few months the novel corona virus spread to several countries transforming an
epidemic quickly into the form of a pandemic. Various researches were done to find an effective way to control the pandemic. Many measures were implemented in all countries all over the world to prevent the spread of the disease. This included shutting down of schools, offices, public places, etc. Lockdowns were implemented which ranged from minor restrictions to locking down of entire countries. “However, while these harsh measures may have been extremely successful in averting catastrophic epidemic rise, they inflict major social and economic expenses on the society at the same time.”(4) Hence, all countries have been trying to find an effective and responsible way to waive off the lockdown gradually. It is possible to achieve this if there is adequate herd immunity being set up in the society. Herd immunity can prevent sudden rebound of new cases during the relaxation period of the lockdown. “The concept of herd immunity is based on the fact that direct effect of immunity in decreasing infectiousness in some individuals can reduce the risk of infection among those who still remain vulnerable in the given population.”(5)

There are two known effective methods to reach herd immunity. The most effective method is to successfully vaccinate individuals. The other method is to let natural immunity generate in individuals naturally infected with the said pathogen. Various mathematical models have been created using statistics of new cases, their recovery rate, overall case fatality rate, data obtained by screening tests, antibody testing in affected individuals, etc. which have helped to learn about the novel corona virus. Mathematical estimates are available which have been used to calculate how we can achieve herd immunity as well as to calculate the risk factors involved in achieving the goal. Studying the mathematical model has been helpful to weigh the pros and cons and to understand whether achieving herd immunity can turn out to be an advantage in this pandemic or prove to be just another unachievable challenge in an already critical situation.

Study Design:

Various mathematical models were studied to understand herd immunity and its significant effect in the COVID-19 pandemic and further observations were hence made. “Herd immunity refers to the indirect protection from a certain pathogen inherited by susceptible individuals when a adequately huge fraction of immune individuals exists in a population.”(6) The concept of classical herd immunity arose from mathematical models that were developed according to the impact of vaccination in a population. Depending on the amount of pre-existing immunity to a certain pathogen in the population at hand, probably due to successful vaccination, addition of a single infected individual to the same population will have varied outcomes. In an entirely susceptible population, the said pathogen will spread through non-immune hosts in a completely reckless manner after exposure of susceptible and infected individuals. However, if a large proportion of the given population is immune to the said pathogen, the odds of effective exposure of susceptible and infected individuals are greatly reduced. If the proportion of vulnerable or non-immune individuals in a population in comparison to infected individuals is too less, then the pathogen cannot spread effectively as the immune individuals prevent its spread and thus the prevalence of the disease will eventually decline. “The point at which the fraction of non-immune individuals falls below the threshold needed for transmission is known as the herd immunity threshold.”(6) It is important to calculate this herd immunity threshold to reach effective herd immunity. The herd immunity threshold, \( h_C \) is calculated as \( h_C = 1 - 1/R_0 \) \(^{(7)}\) \( R_0 \) is the basic reproduction number, defined as the average number of secondary infections caused by a single infectious individual during the early stage of an outbreak in a fully susceptible population.(6,7) Let’s take a pathogen whose value of \( R_0 \) is 5, this suggests that a single infected individual will further infect five other individuals following exposure, assuming a completely susceptible population at hand. The more transmissible the pathogen the greater is the value of \( R_0 \). “\( R_t \) is defined as the number of secondary infections caused by a single infectious individual during the infectious period in a partially immune population.”(6) \( R_t \) is considered a more practical form of \( R_0 \) which uses realistic figures to assess the reproductive number.\(^{(8)}\) “The value of \( R_t \) can be estimated by using the exponential growth method, using data gathered on a daily basis of new COVID-19 cases, or by multiplying \( R_0 \) with the fraction of non-immune population. We can use these estimated values of \( R_t \) to calculate the herd immunity threshold \( h_C \), to cease the spread of infection in the given population. For example, since \( h_C = 1 - 1/R_t \) if the value of \( R_t = 3 \) then, \( h_C = 0.67 \), i.e. at least
two-thirds of the population needs to be immune.”(8) It has been assumed that the value of $R_t$ should be brought down below 1. This can only happen when the immune fraction of the population surpasses the calculated herd immunity threshold level. When this point is reached, the transmission of the pathogen cannot be maintained effectively and there is a significant deterioration seen in the overall cases of infected individuals in the same population.

Although these mathematical models are helpful in making estimates, they majorly rely on assumptions made during creating the models. The model assumes the population to be homogenous in nature, which is far from reality. There are many heterogeneities in a real life population that influence the virulence and transmissibility of the virus. It is also assumed that all individuals in a society will develop absolute immunity to the pathogen and that the immunity developed will have lifelong protection against the pathogen.(6) “A possibility of partial immunity to SARS-COV2, even when it is a new coronavirus, may be present due to some antibody cross-reactivity and a form of partial immunity derived from former infections with the seasonal coronaviruses (OC43, 229E, NL63, HKU1) that have been present in the population for decades, which has already been seen for SARS-COV. A similar case could also be seen for SARS-CoV-2 and might explain the reason why some individuals have milder symptoms or even asymptomatic infections.”(8) This also proves that the virus that affects the population doesn’t have the same effect on all individuals, striking out the possibility of homogenous mixing of the population. A single pathogen will have not a single but numerous values of $R_0$ depending on the communicability dynamics of the virus and the population. To eliminate the assumption of homogeneity, we will have to consider different parameters like age, social activity level, population density, population structure, etc. Some models have been created wherein the population has been divided into different cohorts depending on these parameters to get more accurate values of herd immunity threshold.

Another problem that has emerged depends on the accuracy of screening tests for SARS-COV2 done across countries. If testing is done randomly and assuming that the test has very high sensitivity as well as specificity, we can obtain reasonably accurate estimates of the infected, the population attack rate, and the infection fatality rate. But the current worldwide situation does not bear a resemblance to this ideal condition.(9) If the estimates obtained by testing are low on accuracy, the calculated herd immunity threshold will also ultimately be less accurate. Therefore, it is important to get accurate statistics before making future policies and planning strategies in the ongoing pandemic.

There are various pathways that can be followed to achieve effective herd immunity levels. These pathways are made according to the value of $R_t$ in the given population. If the value of $R_t$ is more than 2, it indicates an uncontrolled epidemic spread of the disease. In this case the best solution would be to limit mortality rates and prevent an epidemic overshoot. A targeted lockdown implemented for a shorter duration once it is observed that we are on the verge of reaching threshold levels, could reduce mortality, and at the same time prevent socioeconomic losses caused by prolonged lockdown measures. If the value of $R_t$ is between 1 to 2, it indicates a controlled epidemic spread of the disease. In these situations, the population can be divided into two groups depending on the risk factors. A lesser degree of social distancing would be implemented to the group with less risk factors while strict measures of social distancing would be implemented to the group with high risk factors to make certain that only the groups with the least risk and least fatality become infected. If the value of $R_t$ is less than 1, it indicates that a local elimination of the disease can be carried out. With values less than 1, pathogen spread cannot be maintained effectively and the epidemic cannot sustain itself. Therefore, the epidemic and be controlled locally. This can be accomplished by early detection of active cases with extensive screening tests, along with strict isolation of primary cases and quarantine of all close contacts to inhibit further spread which will safeguard rapid elimination of the disease.(4)

Although once herd immunity threshold levels are reached by any of these measures, the actual efficiency of herd immunity is still greatly influenced by the strength and duration of the newly acquired immunity. “Recently shared research reports show that neutralizing antibodies were detected amongst patients with mild disease after roughly 10 days, but remarkably neutralizing antibodies were not detected in approximately 30% of patients.”(10) Thus, it can be said that we still do not have
enough research based evidence to find out the definite efficacy of the immunity induced by the pathogen. Herd immunity is extremely effective only when the pathogen induces lifelong immunity, for example measles vaccination. However, this is very rarely seen, as the immunity induced by many other pathogens is either less in strength or diminishes after a while. As a result, herd immunity is not adequately effective and sporadic outbreaks can possibly occur in the population. “Also, if immunity is distributed randomly and unevenly within a given population, masses of vulnerable individuals that habitually contact one another may remain. Even if the fraction of immune individuals in the population as a whole surpasses the threshold, these clusters of susceptible individuals still remain at risk for local spread of infection.”(6)

Most of the models that have been made over the years to learn about herd immunity have considered vaccination as the main source of achieving immunity. However in this case, SARS-COV2 being a new virus in the human population, enough studies have not been carried out yet to develop an effective vaccine. “Taking future research into development of a vaccine into mind, if we vaccinate a fraction $v$ of the population at hand, assuming the vaccine giving 100% immunity, and individuals being vaccinated being selected uniformly, then the reproduction number would now be $R_v = (1 – v) R_0$. Using this, we can derive the critical vaccination coverage $v_c = 1 – 1/R$. So, at the least if this fraction of the community is vaccinated the population will reach herd immunity, as $R_v \leq 1$, and the epidemic cannot sustain itself. However, if the vaccine is less than perfect but only reduces susceptibility by a fraction $E$ (so $E = 1$ corresponds to 100% efficacy), then the critical vaccination coverage is given by $v_c = E^{-1}(1 – 1/R_0)$, inferring that a larger proportion of the community needs to be vaccinated if the vaccine is not nearly perfect.”(7) These calculations may be helpful in testing the efficiency of a vaccine in the pandemic. However, since currently there are so such effective vaccines available for mass production, there is a huge gap that is too difficult to fill to achieve classical herd immunity. Merely naturally induced immunity cannot fulfill this adequately without having harmful consequences. If we rely only on natural immunity, for the herd immunity threshold that needs to be achieved, a large fraction of the population will have to be allowed to be infected by the virus. This can lead to severe rise in mortality rates.

While having the perspective of attaining herd immunity to SARS-COV2, it is mandatory to also keep in mind that there are only finite healthcare resources available throughout countries. As the approach of attaining herd immunity to limit the propagation of the disease has the major consequence of permitting a large portion of the community to be infected, if the healthcare resources are depleted, there would be an escalation in mortality rates not only due to coronavirus disease but also due to all other causes.(6) This would only make the current scenario worse and even more challenging to deal with.

**Results and Discussion**

After studying various models that have been made for achieving herd immunity in the COVID-19 pandemic, it can be said that we do not have enough data or sufficiently accurate data to estimate exactly how much duration it will take to establish adequate herd immunity in the society. It is also not practical to figure out exactly how effective herd immunity can be for this pathogen as we are still in the early phase of the pandemic where sufficient information is still not available to make a valid estimate. The development of a vaccine against the virus can drastically change the situation and may rapidly speed up the process of achieving target herd immunity threshold. But the idea of a 100% effective vaccine inducing 100% immunity in an individual seems too far-fetched. Also, the time taken to create a near perfect vaccine will take years and till then, waiting for herd immunity to be established based on only naturally induced immunity seems inadequate. Without an effective vaccine, there would be unnecessary mortality that accompanies reaching herd immunity which is an unethical compromise. “According to research, the case fatality rate of COVID-19 can be around 0.25–3.0% of the population, proving that the expected number of people who could possibly die from COVID-19, while the population reaches the herd immunity threshold, is too much to accept.”(8)

It is vital now to start planning policies and strategies guided with an end goal in mind. Until now individual countries have been driven by short term targets, such as flattening the curve. The longer term exit strategy should now be considered and should focus on better population
health outcomes as well as the economic outcomes of any action taken. While herd immunity is an approach that has helped in epidemics in previous years, it might not be the best method to follow in the ongoing COVID-19 pandemic considering the countless shortcomings that come along with it. Once we get acquainted better with this new virus and have better research results, as well as an effective vaccine at hand, there is a prospect that achieving herd immunity may perhaps become easier and more effective. But according to the current scenario, achieving herd immunity against the SARS-COV2 virus cannot be made the primary, global objective. Emphasis is given in primary prevention. So basic precautions like hand hygiene (11), avoiding undue public contact through movement and travel (12,13,14) can help a lot to prevent the spread of Covid-19. Also measures need to be taken to reduce depression, anxiety and stress among the general population(15). In its place, making strategies to protect the most vulnerable group of the population should be highlighted, assuming that herd immunity will sooner or later be attained as a consequence of such strategies, instead of waiting in anticipation for the target herd immunity to be achieved.

**Conflict of Interest:** None

**Ethical Approval:** IEC DMIMS, Wardha

**Funding:** DMIMS, Wardha

**References**


Adult Vaccination: The Current Scenario

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Abstract

Vaccination is the process by which a person is made immune to various preventable diseases. Most of the emphasis is given to childhood vaccination and very little focus goes on towards immunizing the adults. Health care services in India has very less contribution to adult vaccination. Hence a desperate need has emerged to develop strategies and schemes for adult vaccination. The disease burden in India is increasing for both communicable and non-communicable diseases. By vaccinating adults against a disease that are known to be life-threatening can reduce the mortality rates significantly. In a country like India with such a huge population, vaccination can be a major intervention in preventing morbidity, mortality, and disability due to many known and preventable diseases. There is no doubt that adult vaccination in India is negligible as protecting adults by vaccination has never been considered as a preventive strategy. Hence there is a need to sensitize the health care providers in our country regarding the same. By vaccinating adults along with children, the chain of spreading the diseases can be broken and quality of life can be improved in a disease-free world.

Keywords: Adult Vaccination, Indications to Use, Guidelines.

Introduction

Vaccination is the process by which a person is made immune to various preventable diseases. Vaccination starts at a very early age, right from birth till old age. However, most of the vaccines given are during childhood and adolescence. (1) Also, most of the emphasis is given to childhood vaccination and very little focus goes on towards immunizing the adults. Vaccinations are important to prevent mortality occurring due to infections that can potentially be prevented. (2-4)

Besides this, the schedule made by the Government of India and World Health Organization for immunization also covers the childhood vaccination with very less focus on adult vaccination. Health care services in India has very less contribution to adult vaccination. Hence a desperate need has emerged to develop strategies and schemes for adult vaccination. The disease burden in India is increasing for both communicable and non-communicable diseases. Framing policies for adult immunization needs infrastructure, strategy, funds, and massive awareness campaign. (5)

By vaccinating adults against a disease that are known to be life-threatening can reduce the mortality rates significantly. This primarily includes communicable diseases such as hepatitis A, hepatitis B, Measles, Mumps, Rubella, Tetanus, Diphtheria, Japanese Encephalitis, human papillomavirus, meningococcus, pneumococcus, typhoid, influenza and others. (5) Hospitalization for diseases as mentioned earlier can be prevented, thus preventing the economical constraints on the patient. The significance lies in the fact that more than 25% of mortality in India is due to infectious diseases. (6)
In a country like India with such a huge population, vaccination can be a major intervention in preventing morbidity, mortality, and disability due to the diseases mentioned above. The most common problems faced in vaccinating adults includes the cost of developing the vaccines for adults. (7) Also, the availability, efficacy, potency, and effectiveness plays a major role in inadequate vaccination. There is no doubt that adult vaccination in India is negligible as protecting adults by vaccination has never been considered as a preventive strategy. Hence there is a need to sensitize the health care providers in our country regarding the same. (5)

Vaccination depends on various factors such as previous vaccination, age, gender, occupation, location, lifestyle, and traveler. (6) Hence by educating an adult regarding the possible diseases he is at risk of developing, he can be motivated to get vaccinated for, preventing any major hospitalization and possible morbidity to the patient. (8)

**VACCINES CURRENTLY IN USE IN INDIA FOR ADULTS INCLUDE:**

Ø **HEPATITIS B:**

- Recombinant hepatitis B vaccine is recommended for all adults. (9)

- At risk population group includes health care workers, people living with HIV positive patients, iv drug abusers, multiple and recurrent blood transfusions, multiple sexual partners, chronic diseases of liver and kidney and sexually transmitted diseases.

- Doses: 1ml (20 mcg) i.m. at 0, 1, and 6 months (STANDARD REGIMEN). But for patients with immunocompromised state and CKD, 4 doses are given at 0, 1, 2 and 6 months as 2ml (40 mcg) i.m.

- The only contraindication includes Hypersensitivity to yeast.

- The effectiveness of vaccines can be assessed by measuring Anti-HBs level, which should be above 10 mIU/ml. if the levels are less than 10 in a normal healthy individual who had received 20 mcg doses, it is recommended to give 3 more doses of 20 mcg at least 1 month apart and retest the levels. If the levels are still suboptimal, then the dose is doubled, that is 40 mcg i.m injections are given at 0, 1, and 6 months.

  - Post-exposure prophylaxis can be given with hepatitis B immunoglobulin within 72 hours of exposure, to be followed by a full course of vaccination. (10-13)

Ø **HEPATITIS A:**

- Inactivated hepatitis A vaccine is available in India.

- A combination vaccine with Hepatitis B is also available.

- API does not recommend Hep A vaccination for all.

- This vaccine is only indicated in high-risk individuals; post-exposure prophylaxis, hemophiliacs and iv drug users.

- It is very important to vaccinate a patient of chronic liver disease, post liver transplant and those waiting for liver transplants as the infection is very severe in such individuals.

- Dose: 2 i.m. doses; the first dose followed by the second dose at 6-18 months. (9)

Ø **DIPHTHERIA, PERTUSSIS AND TETANUS VACCINES:**

- Vaccines available for adults are acellular pertussis (ap), tetanus toxoid (TT), reduced diphtheria with tetanus toxoid combinations (Td) and combination of acellular pertussis, reduced diphtheria and tetanus toxoid (Tdap).

- These are indicated in all adults who did not have any prior immunization.

- All adults up to 65 years can be vaccinated by Tdap (single dose) as per recommendations.

- It is contraindicated in individuals with a history of allergic reactions to these vaccines and also in patients with acute neurological deficits.

- The vaccine used for the pediatric population contains whole cell pertussis, which is contraindicated in adults, as the risk of neurological complications is very high with this vaccine. (14-15)
§ Post-exposure prophylaxis: For any major trauma, TT dose is recommended if the last dose given more than 5 years ago. But in case of minor trauma, TT may not be given if the last dose was within 10 years.

§ In pregnancy, the recommended dose is 3 doses of TT in the second or third trimester with a gap of at least 3 weeks in between. However, if TT or Td is received within 10 years, a single booster immediately after delivery must be given. If more than 10 years have passed, than a single dose is given in the second trimester. (9)

Ø MEASLES, MUMPS, and RUBELLA:

§ It is a live attenuated vaccine.

§ It is given in adults only if the vaccine is not received in childhood.

§ The recommended dose for adults is 2 doses of 0.5 ml s.c. 4 weeks apart.

§ In HIV patients, it can be given if the CD counts are >200 cells/cu.microlitre. (16)

§ Contraindications include fever, pregnancy, history of hypersensitivity and a severely immunocompromised state. (9)

§ Considering teratogenic risk rubella vaccine in particular is avoided in pregnant women and also in those planning a pregnancy should avoid pregnancy after receiving the vaccine for a minimum of 1 month.

§ Current recommendation for any girl ≥15 years is a 3-dose schedule (0, 1-2, 6 months) including younger than 15 years who are immunocompromised and also in HIV infected.

§ This schedule is applied to all types of vaccines (bivalent, quadrivalent, and monovalent vaccines).

§ Rubella is recommended in secondary target populations include females aged ≥15 years or males only if it is affordable and feasible.

Ø VARICELLA AND ZOSTER VACCINES:

§ Both are live attenuated vaccines.

§ It is observed that the incidence of herpes zoster and postherpetic neuralgia is tremendously reduced post vaccination.

§ Varicella vaccine is indicated in patients without prior varicella infections. It is highly recommended in patients of HIV with CD count of >200/cu.microlitre. (16)

§ It is also indicated in post-exposure prophylaxis in adults with no history of infection or immunization. Single dose within 3-5 days post exposure is recommended. (17)

§ Dose: 2 doses (0.5 ml) s.c. four to eight weeks apart.

§ Contraindications to vaccines include pregnancy, hypersensitivity, severe immune compromised host or recent history of blood transfusion.

§ Zoster vaccine is indicated in elderly (>60 years) and individuals with any chronic illnesses. (16)

§ Countries where the varicella infection is acquired in higher age group then average age group can consider alternative vaccination programme. Current recommendations includes a 2-dose schedule as mentioned above.

§ Use of zoster vaccine is not recommended in India as enough evidence are yet to be collected. (17)

Ø PNEUMOCOCCAL VACCINE:

§ It is of two times. One is a polysaccharide vaccine containing 23 serotypes (PPSV23) and another one is conjugate vaccine containing polysaccharide capsules from 13 serotypes (PCV13) bound to diphtheria toxoid.

§ PPSV23 has less immune response but lacks mucosal protection

§ PCV13 is more immunogenic, but also provides good life long immunity. It also protects the mucosal layer. (18-19)

§ CDC guidelines suggest vaccination of adults >65 years of age with single dose PCV13 following which single dose of PPSV23, one year apart.

§ PPSV23 is also recommended individuals aged
<65 years for chronic smokers, and patients suffering from chronic diseases of lung, heart, and liver. \(^{(16)}\)

§ Patients with Immunodeficiency or CSF leak, cochlear implants or asplenia (anatomical or functional) should be given a single dose of PCV13 followed by a single dose of PPSV23, 8 weeks apart.

§ In patients posted for elective splenectomy, a single dose of PCV13 must be given 2 weeks before surgery followed by single dose PPSV23 after 5 years. \(^{(16)}\)

Ø Meningococcal Vaccine:

§ Two types of vaccine are available – polysaccharide and conjugate vaccines.

§ Polysaccharide vaccines are of two types – bivalent and quadrivalent.

§ Conjugate vaccine is quadrivalent. In comparison to the polysaccharide, it is more immunogenic. Its protection starts 28 days post vaccination. It is not recommended for individuals above the age of 55 years.

§ Group B meningococci are not covered under any of the vaccines mentioned above. \(^{(20)}\)

§ This vaccine is indicated in health care workers, contact with cases, overcrowded places, immunodeficient patients and asplenia (anatomical or functional).

§ In the case of late complements (C8, C9) deficiency, the pneumococcal vaccine is recommended as these patients are at risk of developing an infection.

§ As per Indian national policy, MPSV4 vaccine is given to all Haj Pilgrims.

§ Dose: 0.5 ml s.c. a single dose of injections. For patients with asplenia, 2 doses are recommended 8 weeks apart.

§ Cautious use is advised in patients with a history of Guillain-Barre Syndrome. \(^{(9)}\)

Ø Hemophilus influenzae Vaccine:

§ Two types of vaccines are available.

§ One is trivalent (TIV), another one is a live attenuated virus (LAIV).

§ These vaccines protect after 2 weeks of injection.

§ It is indicated only for at-risk population, viz, old age (>50 years), chronic kidney and liver conditions, asplenia, diabetes mellitus, pregnancy, health care workers and immunocompromised host and transplant recipients.

§ It is contraindicated in patients with history of Guillain-Barre Syndrome and hypersensitivity.

Ø LAIV is not used in India. \(^{(17)}\)

§ Dose: TIV is used as a single dose of 0.5 ml i.m. \(^{(9)}\)

Ø Human Papillomavirus Vaccine:

§ Two types of vaccines are available – Gardasil (quadrivalent) and Cervarix (bivalent).

§ Gardasil is active against HPV 6, 11, 16 and 18 subtypes while Cervarix has its action on HPV 16 and 18.

§ It is indicated in all females of age between 13-26 years and males 13-21 years.

§ Dose: 3 doses at 0, 2 and 6 months. \(^{(16)}\)

§ It is contraindicated in pregnancy.

Ø Japanese Encephalitis:

§ It is a live attenuated vaccine.

§ Dose: it is given a single 0.5 ml s.c. dose. Followed by a booster dose after at least one year.

§ JE vaccine is currently not recommended for adults. \(^{(16)}\)

Ø Yellow Fever Vaccine:

§ It is indicated only in individuals traveling to endemic countries and under the age of 60 years.

§ Dose: 0.5 ml s.c. single dose. A booster dose is to be given every 10 years.

§ Such individuals are issued YELLOW CARD that is valid after 10 days of vaccination until 10 years after which booster dose is indicated. \(^{(17)}\)
Ø TYPHOID:

§ Two types of typhoid vaccines are available.

§ One is live oral Ty21a vaccine, an enteric coated capsule

§ Another one is an injectable Vi capsular polysaccharide antigen vaccine (Vi CPS). Efficacy is almost equal for both these vaccines. (21)

§ Typhoid vaccines are not recommended currently as a routine vaccination for adults.

§ It is indicated only in cases where individuals are in contact with known S. Typhi carriers.

§ Dose: Oral Ty21a is given in 3 doses, alternate day followed by booster dose every 3 years. Vi CPS is given a single dose of 0.5 ml i.m. with a booster given after every 3 years.

§ Contraindications include: immune compromised host, pregnancy and children (<6 years) for oral vaccine and <2 years age for Vi CPS.

Ø CHOLERA:

§ It is an oral vaccine.

§ It is not a recommended vaccine for adults. (16)

NEVER VACCINES:

· DENGUE:

o Dengvaxia (CYD-TDV), has been licensed and five additional dengue vaccines are in clinical development with 2 in Phase III trials. Dengvaxia is given in three doses of i.m. schedule at 0, 6 and 12 months.

o WHO-SAGE recommends that users should be limited to areas where the disease is common because vaccination may increase the risk of dengue fever in people who have not been previously infected with the dengue virus. (22)

· MALARIA:

o At present, there are no approved vaccines for malaria.

o Mosquirix (RTS, S/AS01) acts against P. Falciparum and is the most promising vaccine. It requires 4 doses with the efficacy of 26-50%. (23)

· HEPATITIS E:

o At present, WHO has made no recommendation on the use of this vaccine. (24)

· HIV/AIDS:

o VaxGen gp120 protein subunit vaccine is in phase III trial at present. No significant results are achieved till now. (25)

Apart from the forementioned vaccines, adult vaccination remains one of the most untouched modality of health care in India. There is no doubt that childhood vaccination has increased the longevity of life. But the quality of life deteriorates as the age progresses as the forehead mentioned diseases commonly manifest in adults and are one of the common infectious causes leading to mortality, morbidity and poor quality of life amongst old age people. (21)

Not only in India, but adult vaccination remains an untouched modality even in developed countries. But steps are now been taken towards it in developed countries. This is an alarm for a country like India, where poverty, overpopulation, unhygienic conditions prevail so much that communicable diseases spread very fast. (26)

CURRENT INDICATIONS AND GUIDELINES FOR USE OF VACCINATIONS IN ADULTS:

Vaccines received in childhood plays an important role in deciding the vaccinations required for an adult. The vaccinations in healthy adults differ from an individual to other.

Major guidelines followed in India are: (Details mentioned in the table given below; table 1)

· API (Association of Physician of India) – Expert panel guidelines.

· WHO (World Health Organization) guidelines.
Table 1:

<table>
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<tr>
<th>API Guidelines</th>
<th>WHO guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vaccinations for all healthy adults:</td>
<td>Vaccinations for all healthy adults:</td>
</tr>
<tr>
<td>a. DPT</td>
<td>a. typhoid</td>
</tr>
<tr>
<td>b. MMR</td>
<td>b. Rubella</td>
</tr>
<tr>
<td>c. HPV (9-26 years)</td>
<td>c. Tick borne encephalitis</td>
</tr>
<tr>
<td>d. Influenza (&gt;50 years)</td>
<td>d. Hepatitis A</td>
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<tr>
<td>e. Zoster (&gt;60 years)</td>
<td>e. Meningococcal</td>
</tr>
<tr>
<td>f. Pneumococci (&gt;65 years)</td>
<td></td>
</tr>
<tr>
<td>2. Vaccinations for Health care workers:</td>
<td>Vaccinations for at risk populations (Health care workers):</td>
</tr>
<tr>
<td>a. Hepatitis A</td>
<td>a. BCG</td>
</tr>
<tr>
<td>b. Hepatitis B</td>
<td>b. Hepatitis B</td>
</tr>
<tr>
<td>c. HiB</td>
<td>c. Polio</td>
</tr>
<tr>
<td>d. Varicella</td>
<td>d. Diphtheria and Pertussis</td>
</tr>
<tr>
<td>e. Rabies</td>
<td>e. MMR</td>
</tr>
<tr>
<td>f. Cholera</td>
<td>f. Meningococcal</td>
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<tr>
<td>g. Typhoid</td>
<td>g. Influenza</td>
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<td>h. JE</td>
<td>h. Varicella</td>
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<td></td>
<td>i. Rabies</td>
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<td></td>
<td>j. JE</td>
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</tbody>
</table>

Other guidelines include The Advisory Committee on Immunization Practices (ACIP) guidelines from Centers for Disease Control and Prevention.

**Conclusion**

By vaccinating adults along with children, the chain of spreading the diseases can be broken and quality of life can be improved. It is high time that government and other health service providers make a call regarding adult vaccination and frames new guidelines, keeping in mind the education level, the requirement of a particular vaccine in an individual, socioeconomic status of an adult and providing utmost immunization to the patient thereby preventing major diseases. The government needs to frame a proper guideline and implement it by promoting it and creating awareness among the general population.

Vaccinations Aren’t Just For Children.

Adults Need Them Too!

**Conflict of Interest:** Nil

**Source of Funding:** Nil

**Ethical Clearance:** Obtained from Institutional Ethical Committee, DMIMS, Wardha.

**References**


5. API Guidelines “Executive Summary; The Association of Physicians of India Evidence-Based Clinical Practice Guidelines on Adult Immunization” Expert Group of the Association of Physicians of India on Adult Immunization in India JAPI. 2009;57:345-56


Correlation between Gestational Age and Abdominal Circumference in Second Trimester

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Abstract

Gestational age is age of unborn baby, defined in weeks as beginning from first day of last menstrual period prior to conception. Estimation of gestational age and thereby forecasting Expected Date of Delivery is not only concern of the Individual but it is invaluable in the diagnosis of intrauterine growth retardation of fetus and obstetric planning. Determination of age of a fetus by using various methods is required for medico legal purposes in both civil and criminal matters. In present study we assesses Correlation between gestational age and abdominal circumference in second trimester, The study of Correlation between gestational age and abdominal circumference in second trimester by Ultrasonography was carried out and collected data was tabulated according to weeks of menstrual cycle and were taken in centimeters. Standard deviation of abdominal circumference for each week was calculated. We found that Abdominal Circumference is statistically highly significant and the regression equations derived for growth parameter for estimating gestational age in a normally developing fetus, increase with gestational age, showed good correlation with gestational age.

Keyword: Gestational age, abdominal circumference, age determination, Ultrasonography, Second trimester.

Introduction

Human development starts as oocyte from female which is fertilized by the sperm. Cell division, differentiation, growth transfigure the fertilized oocyte into a multicellular adult human being. Most of the changes occur during the early fetal and embryonic period, the development of which divided into pre and postnatal period. There are many changes that occur from the 3rd to 8th week called as embryonic development and changes occur from 9th week to birth into a recognizable human being called a fetus.¹ 

Gestational age is age of unborn baby, defined in weeks as beginning from first day of last menstrual period prior to conception. Trimester is period of three calendar months during a pregnancy. Radiologically the period of gestation is grossly divided into three trimesters. Estimation of gestational age and thereby forecasting Expected Date of Delivery (EDD) is not only concern of the Individual but it is invaluable in the diagnosis of intrauterine growth retardation of fetus and obstetric planning.² 

The parameters either singly or in combination useful in predicting the gestational age with fair degree of accuracy are Naegeles formula, Date of quickening, Palpation of fetal parts and Auscultation of fetal heart sound.³ 

The methods like physical examination, menstrual history, and laboratory methods have limitations in assessing fetal maturity, development and well being. At the same time Roentgenography like procedures having
hazards of invasive procedure or radiation compelled the research of safer, non-invasive and reliably predictive investigation modality, it was brought forth in the form of Ultrasonography.4

Determination of age of a fetus by using various methods is required for medico legal purposes in both civil and criminal matters.5, 6

Ultrasonography is non-ionising, non-invasive, safe and accurate method of objectively evaluating the fetal growth in uterus. In any obstetrics case correct assessment of gestational age is keystone. Measurements and fetal characteristics are helpful in estimating fetal age.

To determine the fetal age at the end of 1st trimester the crown rump length is method of choice because of negligible variation in the size of fetus during the period. In second and third trimester, fetus grows sufficiently in size; several structures can be identified and measured ultrasonographically.

Accurate knowledge of age is a keystone in the obstetrical and medicolegal Aspect legally also helpful in successfully managing the antepartum care of the patient and is critically important in the interpretation of antenatal test and successful planning of appropriate therapy and interventions.7, 8

Materials and Methods

The study Correlation between gestational age and abdominal circumference in second trimester was carried out at Govt. Medical College and Hospital, Nanded, between July 2011 to July 2013 period. The study included 150 females attending ANC clinic for Ultrasonography screening at Medical College and Hospital. Subjects of the study mainly include urban as well as rural areas in the vicinity.

Include Criteria

Women with known LMP

Women with regular menstrual cycle

Women with singleton and uncomplicated pregnancy

Women having age between 18 and 34 yrs.

Exclusion Criteria

Women with multiple pregnancies

Women with irregular menstrual cycles

Women having diabetes mellitus

Women with diseases like hypertension, chronic renal disease, heart diseases, iron deficiency anemia. Women having Fetus with congenital anomalies.

For collection of the data proper permission was obtained from ethical committee and radiology department. In this study various particulars of the subjects like age, menstrual and obstetric history had been recorded in the Proforma.

The American Institute of Ultrasound in Medicine recommendations were used for measurements of all the fetal parameters.9

The fetal Abdominal Circumference (AC) was measured at the skin line on a true transverse view at the level of the junction of the umbilical vein, portal sinus and fetal stomach. The fetal abdomen was identified with the help of digitaliser.

Date of Ultrasonography of subject is recorded and Gestational age of the fetus in terms of weeks was calculated from last menstrual period in the Proforma. The data so collected was then subjected to statistical analysis by expert statistician with the help of SYSTAT Crainsoft version 12 software. Standard statistical methods, parametric methods were used for the evaluation and significance.

Results

The study of Correlation between gestational age and abdominal circumference in second trimester by Ultrasonography was carried out at Govt. Medical College and Hospital Nanded.

The collected data was tabulated according to weeks of menstrual cycle and were taken in centimeters.

Standard deviation of abdominal circumference for each week was calculated. The mean of each parameter calculated statistical for each week. The completed week considered as the week of gestation. For e.g., 13th week refers to 13.00 to 13.86 weeks of menstrual age. 7 days =
1 week, hence 1 day = 0.14 weeks. Like this subsequently for each day.

Ultrasongraphic Abdominal Circumference was measured in a total of 150 subjects. The observations of week wise mean values and standard deviation of fetal Abdominal Circumference are shown in (Table 1).

Table 1: Mean and Standard deviations of fetal Abdominal Circumference (Week wise).

<table>
<thead>
<tr>
<th>Menstrual age in weeks</th>
<th>No. of cases</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
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<tbody>
<tr>
<td>13</td>
<td>8</td>
<td>8.35</td>
<td>0.73</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>8.9</td>
<td>1.13</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>9.92</td>
<td>0.84</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>11.0</td>
<td>1.07</td>
</tr>
<tr>
<td>17</td>
<td>12</td>
<td>12.08</td>
<td>0.76</td>
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<tr>
<td>18</td>
<td>9</td>
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<td>20</td>
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<td>22</td>
<td>12</td>
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<tr>
<td>28</td>
<td>9</td>
<td>22.04</td>
<td>1.44</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>150</strong></td>
<td></td>
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</tbody>
</table>

Regression output for 2nd trimester (13 to 28 weeks)

Regression equation:

$$G.A = 6.36 + 0.956 \times AC$$

From the above equation it is clear that during the second trimester, for every 1 cm increase in AC, the gestational age (G.A) increases by 0.956 weeks.

As the value of R is 0.9451 the variation in fetal growth on the basis of Abdominal Circumference during second trimester can be explained to the extent of 94.51%.
The value of R is highly significant (Student’s ‘t’ test value = 113.04, p<0.0001, very highly significant) showing that there is statistically highly positive or strong positive association between Gestational age and Abdominal Circumference.

Discussion

Ultrasonography is key imaging technique in the assessment of fetal growth because of its low cost, availability, and without any adverse effects. Ultrasonography can detect the fine observations of the chorionic sac and its contents during the various stages of fetal and embryonic period. Along with this technique can also detect anomalies abnormality various presentations related fetus at a very early stage. Therefore various advances in Ultrasonography have made this technique a crucial tool for prenatal diagnosis which is a most reliable method for the growth of the fetus.

It is observed that upper extremities almost reach to development by the end of 12th weeks, compare to lower extremities. Appearance of primary ossification centre for cranium and long bones develop by the end of 12 weeks. Various ossification centers can be observed during this period along with bones on Ultrasonography. Fetal abdominal, body and extremity measurements have been widely reported and found to be used in second and third trimester.10

After 10th week of gestational period one can differentiate soft and hard tissues after which measurement of various parameters like abdominal circumference other Parameter can be done by Ultrasonography which can be recommended.

The differentiation between hard and soft tissues of the embryo is possible after about 10th week of gestation when other parameters like Abdominal circumference, length of long bones can be measured and become more important than the crown rump length. Measurements of fetal parts during routine Ultrasonography screening have been recommended.11

Tamura RK et al found that fetal abdominal circumference added another dimension to the interpretation of cephalic growth particularly in indentifying macrosomic fetuses. The study also revealed that AC was significant in assessment of fetal size in those, who were for obstetric reason had to be delivered in the latter part of second trimester or early third trimester.12

Juozas K et al Found linear correlation of gestational age with abdominal circumference .13 Similarly Jain ND et al found that parameters increased progressively through the 2nd and 3rd trimester. These parameters were reliable indicators of menstrual age.14

Johsen SL et al Analysed that abdominal circumference at 10–24 Weeks of gestation. And the effect of fetal size at 10–24 weeks of gestation on pregnancy duration was assessed. Fetal size in the second trimester is a determinant of birth weight and pregnancy duration support a concept of individually assigned pregnancy duration according to growth.15

Bhusari PA et al found that Second trimester Abdominal Circumference Shows linear growth as gestational age advances and are strongly correlated with each other. The study was comparable to other studies carried out on aborted fetuses taking actual measurements. Thus accuracy and reliability of ultrasonographic measurements was established.16

Conclusion

Sonographically measured parameters during second trimesters of pregnancy were subjected to statistical analysis by simple linear regression. The regression was done separately for each parameter and for each week. Abdominal Circumference is found to be statistically highly significant. The regression equations derived for growth parameter for estimating gestational age in a normally developing fetus, increase with gestational age, showed good correlation with gestational age. Assessment of gestational age helped in calculating the EDD (expected date of delivery) in all patients, thus improving the antepartum management. Gestational ages are fairly accurate predictors of fetal growth. In Present study, in second trimester abdominal circumference is the sensitive parameter and results of present study was comparable with previous studies.

Ethical Clearance: Taken from institutional ethical committee.

Funding: Article did not receive any specific grant
Conflict of Interest: Author declares that there is no conflict of interest

References


Nursing Perspectives in Diabetic Ketoacidosis

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Abstract

Diabetic ketoacidosis (DKA) is a serious life threatening condition and it occurs when insulin production in our body is less. Diabetic ketoacidosis is a complication of diabetes mellitus and it mostly occur in hospitalized patients. Diabetic ketoacidosis condition mostly found in both type of diabetes such as Type 1 and Type 2 diabetes patients. In between 20-30% of cases of diabetic ketacidosis condition occur in newly diagnosed patient with diabetes. Almost till 20 years the mortality rate of diabetic ketoacidosis is 100 percent and till now there is no reduction in prevalence of diabetic ketoacidosis in younger patients due to that children are continuous suffering with death.

The mortality rate in Diabetic ketoacidosis is very important because it is a very serious life threatening condition so the prevalence of diabetes is very crucial. The younger are the foundation of future health care delivery system so the evaluation of their knowledge and continue development and upgrading regarding awareness and understanding the sign and symptoms of diabetic ketoacidosis it helps to reduce the mortality rate of Diabetic ketoacidosis.

It’s an emergency condition, so the health care professional becomes a vital part of managing diabetes-related hyperglycemic emergencies, rising health care costs and changing health care systems preventing signs and symptoms of diabetic ketoacidosis remains an essential requirement.

Effective management and prevention on diabetic ketoacidosis can reduced longer stay time in hospitalized patient.

Keywords: Nursing perspective, Knowledge, Prevention, Sign and symptoms, Diabetic ketoacidosis.

Introduction

Diabetic ketoacidosis is also called as diabetic acidosis and it is a serious life threatening complication of diabetes mellitus which causes a serious problem. Mostly the diabetic ketoacidosis condition affects to insulin dependent diabetes patients but it also affect to Non – insulin-dependent diabetes patients.¹ Diabetic ketoacidosis is a major diabetes complication in the United States and internationally.² Diabetic ketoacidosis is an metabolic emergency and it occurs with insulin deficiency, hyperglycemic, ketonemia and acidosis patients with diabetes mellitus.³

Diabetic ketoacidosis occurs when insulin production is less and severe insulin resistance in the hyperglycemic state. The crises occurs when there is problems with production and release of insulin. Dysfunction of beta cells results from inadequate glucose level stimulate to secrete amount of insulin and ultimately elevation of blood glucose level occur after a digestion of meal and with this formation of new glucose is suppressed. But in a hungar state, glucagon (counter-regulatory hormones) in which stored material are properly activated and formation of glucose is elevated.² Diabetic ketoacidosis disease was identified by Dreschfeld in 1886 until 1922 insulin was discovered. Since 20 years the mortality rate of diabetic ketoacidosis was 100 percent and there is no reduction in mortality rate till now.

The approximately 20 -30% cases of Diabetic ketoacidosis occur in recently diagnosed patients with diabetes. The mortality rate of diabetic ketoacidosis is
occur due to its complication such as one of the cerebral edema. The knowledge regarding diabetic ketoacidosis that helps to adult and youth for the adoption of good health-related practices. Youth and adults are the pillars of our future health care system and the evaluation and continuous improvement of their understanding is very necessary and understanding of signs and symptoms of Diabetic Ketoacidosis and help to reduce the high mortality rate.  

It is an emergency condition, and the health care provider is a critical part of handling Hyperglycemiasis. Similar diabetes emergencies, with higher health care charges and prevention of signs and symptoms of diabetic ketoacidosis remains an essential requirement for a changing healthcare managing and avoiding diabetic ketoacidosis in hospitals, admissions can be reduced and stay time shortened.

PREVALENCE OF DIABETIC KETOACIDOSIS

Prevalence and occurrence of diabetic ketoacidosis and its symptoms

Very few studies have examined the incidence or prevalence of diabetic ketoacidosis in patients with diabetes, the Danish National Patient Registry identified four thousand eight hundred and seven admissions in 1996–2002 and 137 deaths diagnosed with diabetic ketoacidosis in 1996–2000. The estimated annual incidence of diabetic ketoacidosis was 12.9 per 100,000, higher in males relative to females over the age of 40 years. And the prevalence of diabetic ketoacidosis over 50 years in type 2 diabetes is approximately 12 percent of all patients.

Compared to previous studies, the occurrence of incidence of diabetic ketoacidosis in children is decreasing with a high mortality rate recently and younger. Diabetic ketoacidosis is a type 1 diabetes metabolic syndrome typically characterized by acidosis, ketosis, and hyperglycemia. Diabetes symptoms that may cause DKA to develop include polyuria, polydipsia, polyphagia, weight loss, vomiting, abdominal pain and fatigue.

PRECIPITATING FACTORS IN DIABETIC KETOACIDOSIS

Precipitating factors such as infection, insulin omission or inadequate insulin administration, acute diseases include the cardiovascular system (myocardial infarction, stroke) and gastrointestinal tract (blindness, pancreatitis), including endocrine diseases (Cushing’s syndrome, acromegaly) and Stress due to recent surgical procedures may develop DKA which causes dehydration and may increase insulin hormones. Medicinal products such as corticosteroids, diuretics, beta-blockers, antipsychotics, and antiepileptics that influence carbohydrate metabolism and may, therefore, precipitate DKA. Many factors may also lead to DKA, such as eating disorders, psychiatric issues, insulin pump failure and illegal use of the drug.

SIGN AND SYMPTOMS OF DIABETIC KETOACIDOSIS

Diabetic ketoacidosis is buildup acid in blood it occurs when the blood sugar level rises it is life-threatening but it takes a long time to become serious so it can prevent or treat in early. It causes When the body isn’t producing enough insulin. And body cell doesn’t use sugar from the blood it uses fat as fuel energy and burn the fat and make acid called ketones.

Those who have type 1 diabetes can develop ketoacidosis and Diabetic ketoacidosis happens with type 2 diabetes also and it leads to severe dehydration. When the blood sugar level rises above 240mg /dl have symptoms like dry mouth, feeling of thirsty, so check for ketone.


LABORATORY INVESTIGATIONS AND DIAGNOSIS CRITERIA OF DIABETIC KETOACIDOSIS

Initial laboratory examination of patients includes Plasma glucose, blood urea nitrogen, creatinine, electrolytes, osmolality, serum and urinary ketones, and urinalysis, as well as initial blood gases and a full differential blood count. It should also be obtained an
electrocardiogram, X-ray in the chest, and samples of urine, sputum or blood. The DKA diagnosis includes Hyperglycemia (larger than 250 mg / dL), ketosis (anion gap greater than 12 mEq / L) and acidosis (arterial pH smaller than or equal to 7.3). The intensity of DKA is classified as mild, moderate, or extreme, depending on the magnitude of the decrease in levels of arterial pH, serum bicarbonate, and emotional, rather than hyperglycemic. Not all ketoacidosis patients do have DKA. Medical history and plasma glucose levels range from slightly elevated (rarely 200 mg / dl) to hypoglycemia, differentiate between malnutrition ketosis and alcohol ketoacidosis.  

**TREATMENT STRATEGIES**

The DKA treatment target involves the preservation of volume status, hyperglycemia and ketoadosis, metabolic abnormalities, electrolyte abnormalities, and precipitating factors. Before beginning a physical test, diabetic ketoacidosis care should be done first. Basic parameters should be obtained, and the final diagnosis should be made.

1. **Fluid therapy in DKA**: Replacement of fluid with normal saline also recommended to help expand interstitial, intracellular, intravascular and perfusion of kidney. Lacking cardiac compromise: First few hours of treatment 0.9 percent NaCl ie Isotonic saline should be administered intravenously at 15–20 ml / kg/1 h or 1–1.5 liter. But in general, if 0.45 percent NaCl infused at 250–500 ml / his elevated or normal at that time, serum sodium is. If the corrected Serum sodium is low, 0.9 percent NaCl should be intravenously administered at 250-500 ml / h. To know the successful progression with fluid replacement, blood pressure monitoring, and fluid deficiency are expected to correct within 24 hours of treatment. Measurement of the input/output fluid, laboratory values, should be carried out as required. To avoid iatrogenic fluid overload in renal or heart compromised patients, monitoring should have been updated for serum osmolality, frequent renal evaluation, assessment of cardiac status, evaluation of mental status. Fluid replacement administration depends on hemodynamic status, Status of hydration, serum electrolyte levels and urinal output. If the glucose level falls below 250 mg/dl, NaCl with DNS should administer and insulin administration continued until ketonemia is controlled.

2. **Insulin administration Therapy**

   The main objective of DKA treatment is to administer insulin on regular basis (0.1 units/kg) followed by (0.1 units/kg / h) by continue administration of intravenous infusion or repeated use of subcutaneous injection. If the glucose value in the first hour does not decrease by 50-75 mg, the dose of insulin infusion should increase for each hour until normal glucose levels are achieved.

3. **Potassium**

   Between mild to – moderate range of hyperkalemia is common in DKA patients. Acidosis correction, insulin administration therapy, volume expander helps to reduce the concentration of potassium in the serum. Replacement of Potassium should be initiated to prevent hypokalemia when the Serum rates fell below normal. Treatment purpose is to keep the serum potassium levels within the normal range of 4–5 mEq / l. In general, DKA patients can develop hypokalaemia. In these cases the treatment of potassium will start with fluid therapy replacement and insulin therapy should be put off until potassium restore concentration to > 3.3mEq / l to avoid arrhythmias and weakness in the respiratory muscle.

4. **Bicarbonate**

   The use of sodium bicarbonate at DKA is only recommended if the pH value is below 7. When the patient is pH<6.9 then inject 100mmol of sodium bicarbonate (two ampules) with 20mEq KCl in 400ml of sterile water (an isotonic solution) for 2 hours at a rate of 200ml / h until the venous pH is > 7.0. Repeat until pH reaches > 7.0 every 2 hours if pH remains < 7.0.

5. **Phosphate**

   If DKA’s total body phosphate deficiency is 1.0 mmol / kg body wt on average, serum phosphate levels are often normal or increased. For patients with heart disease, anemia or breathing disorder and with concentration of serum phosphate < 1.0 mg / dl, 20–30 mEq / l. Treatment of deficiency of potassium phosphate should be used to replace fluids at a rate of 4.5 mmol / h (K2 PO4 1.5 ml / h). The average replacement rate of phosphate considered safe is 4.5 mmol / h which helps to prevent hypophosphatemia-related heart and skeletal muscle weakness and respiratory failure.
Complication Associated with DKA

DKA presents the two most common complications, hypoglycemia and hypokalemia, but these complications occurred with the low-dose insulin therapy. In DKA Constant monitoring of blood glucose (every 1–2 h) is required because many patients may experience hypoglycemia during DKA treatment without sweating, nervousness, fatigue, nausea, and tachycardia manifestations. For adult patients, cerebral edema is particularly rare when being treated with DKA. Cerebral edema symptoms include headache onset, gradual awareness deterioration, seizures, looseness of sphincter muscle incontinence, pupillary changes, bradycardia, blood pressure elevation, and respiratory arrest. Other complications of DKA include Syndrome of an adult respiratory syndrome (ARDS), and hyperchloremic acidosis, non-anion gap acidosis in hyperchloremic. In DKA patient Rhabdomyolysis in the compartment that causes the syndrome can lead to acute renal failure, severe hyperkalemia, hypocalcemia and muscle swelling. Classical signs of rhabdomyolysis include myalgia, fatigue and dark urine. It is recommended that the creatine kinase concentrations be detected early every 2 to 3 hours.  

Preventive Strategies

Diabetic ketoacidosis in a patient who is newly diagnosed with diabetes can only be prevented if the general public and primary care physicians are aware of the symptoms and if doctors are alert to the possibility of developing diabetic ketoacidosis, particularly concerning young children. It is quick to do a urine test for glycosuria. Adequate education and support will prevent diabetic ketoacidosis in patients with proven diabetes (and for their families). Identification of children at risk for such behaviors and social and psychological support intervention can alleviate these issues. Given the risks and associated symptoms and complications of in patients with Diabetic ketoacidosis, strategies to prevent infections effectively are of paramount importance. Continued patient self-management awareness and support are vital for avoiding acute complications and reducing the risk of chronic complications. Patient care is the key to learning to fight illness.

The four important points are:

1. To explain the importance of insulin therapy during illness, and to emphasize that insulin should never be interrupted.
2. Regular interaction with health-care professionals is initiated.
4. Ensuring adequate intake of liquids

Preventive strategies include avoiding excessive hydration and rapid plasma osmolality reduction, decreasing serum glucose levels and maintaining serum glucose concentration from 250 to 300 mg / dl to normal serum osmolality and improving mental status. Cerebral edema care requires Mannitol injection, which should be recommended for mechanical ventilation.

Prognosis

Many studies suggest that the prognosis of properly treated diabetic ketoacidosis patients is excellent, particularly in the younger patients. The average mortality rate for DKA is 2 per cent or less. The indices of weak DKA prognosis are deep coma, hypothermia, and oliguria. The worst prognosis is typically seen in older patients with serious undercurrent diseases (e.g. myocardial infarction, sepsis, or pneumonia), particularly when these patients are treated outside an intensive care unit.

Discussion

The many studies revealed that the majority of participants exhibited average levels of knowledge about diabetic ketoacidosis. On the other hand, regarding awareness, there was minimum awareness concerning the causes, signs and symptoms and complications of DKA. However, this study revealed some gaps in the awareness concerning the prevention of sign and symptoms plan of management and complications of DKA. It has been concluded from the maximum study that the minimum of the individual has a basic knowledge of DKA and its management except in certain points; the importance of the collection of blood for a metabolic profile before initiation of therapy, stopping of insulin when the patient becomes hypokalemic, fluids infusion should be continuing for 24 hours, The importance of hourly blood glucose monitoring in patients and the use of sodium bicarbonate is controversial and recommended only when PH is below 7. There was a lack of DKA
awareness among participants concerning complications associated with DKA, diagnostic criteria and to some extent the precipitating factors. The overall knowledge and awareness related to the prevention of signs and symptoms were average among diabetes patients.  

**Conclusion**

With the combination of interdisciplinary collaboration and coordinated care, DKA’s mortality has decreased dramatically in recent decades. Current practitioners face problems including monitoring DKA patients at the correct level of treatment and educating patients to stop repeating DKA episodes. The potential cost savings in the medical floors associated with caring for less critical DKA patients must be balanced against staffing ratios. Prevention of DKA may require further research and cooperation among hospitalized and outpatient practitioners, as well as patient education.  

**Ethical approval :** Nill

**Conflict of Intrest :** The author declares that there are no conflicts of intrest.

**Funding :** Self

**References**

Knowledge, Attitude, and Practice Regarding Child Abuse and Neglect among Dental Practitioners

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Abstract

Background: Child abuse and neglect (CAN) is a prevalent and growing public health problem. Dentists are in a strategic position to identify and report cases of CAN as common signs of child abuse are in the orofacial region. Proper knowledge and awareness are required for early identification and intervention to prevent untoward consequences.

Methodology: A cross-sectional observational study using a predesigned three parts questionnaire was conducted among general and specialty practitioners in branches of dentistry of Dakshina Kannada district, Karnataka to assess the knowledge, awareness, and behavioral practice towards child abuse and neglect. The data extracted were tabulated, statistically analyzed and results calculated based on percentages using SPSS.

Result: The participants in the current study demonstrated sufficient knowledge of the signs and symptoms of child physical abuse, actions that should be taken in suspected cases, circumstances in which to report such cases, and the legal authorities to which they should be reported. Most of the participants desired for further training such as courses or workshops on examining, diagnosing, and reporting cases of child abuse.

Conclusion: - The study suggests that dental practitioners need an effective education to increase their knowledge and awareness on CAN for its precocious identification and diagnosis as an assay for early intervention.

Keywords- Child abuse, child neglect, reporting, child maltreatment, knowledge.

Introduction

Child abuse and neglect is an augmenting element affecting the community in the present scenario. Since face and oral cavity are often the prime focus of child abuse it becomes the authority of dentists to acquire knowledge to diagnose and report child abuse and neglect cases. WHO estimates that nearly 3 in 4 children of age 2–4 years regularly suffer physical punishment and/or psychological violence at the hands of parents and caregivers. ¹

The short and long-term consequences of victims of CAN have been well documented. Well-established empirical evidence shows a ‘graded’ relationship between the number of unfavorable childhood maltreatment exposures and the occurrence of depression, poor academic performance, and even suicidal ideation among youth.²

Studies have reported that orofacial trauma is present in approximately 50 to 75 percent of all reported cases of physical child abuse.³ This high frequency of
orofacial lesions associated with child abuse place the dentist in the front line to individualize and to intercept an abused child.

As per the available literature, only 1% of child abuse cases were reported by dental professionals which draws attention to reasons behind underreporting. Analysis of research from different parts of the world has indicated that healthcare providers fail to report suspected cases of abuse, mainly due to a lack of knowledge. As Dentists are in a unique position that allows them to identify and report abuse cases, their knowledge and attitudes are essential factors in fulfilling this obligation. To the best of our knowledge, studies have not been conducted in this area to investigate the recognition and reporting of child abuse by dentists. Thus a questionnaire-based study was conducted among dentists practicing in Dakshina Kannada district to assess their knowledge, attitude, and practice regarding child abuse and neglect.

**Methodology**

The study was conducted among general dentists and specialty practitioners of Dakshina Kannada district, Karnataka, India to evaluate the knowledge, attitude, and practice regarding child abuse and neglect. Ethical approval was obtained from the Institutional Ethical Committee of KVG Dental College & Hospital, Sullia. (IECKVD/FR07/2019-20)

Validity and reproducibility test of the questionnaire were conducted with five faculty members of the institution to evaluate understanding, validate the content and attribute scores to each question accordingly:

- 0 (I did not understand anything),
- 1 (I understood just a little),
- 2 (I understood it in part),
- 3 (I understood almost everything, but had some doubts),
- 4 (I understood almost everything),
- 5 (I understood it perfectly and had no doubts).

Scores 0, 1, 2, and 3 were rated as insufficient understanding, and scores 4 and 5 as sufficient understanding, as suggested by Conti et al.\(^4\) One question was eliminated, two were corrected and the remaining were found to be appropriate. The finalized questionnaire written in English consisted of three sets of questions- the first set included 14 questions related to knowledge (table 1), the second set included 2 questions related to the attitude of the dentist (table 2) and third set included 5 questions related to practice they followed. (table 3). 20/21 questions in the study were dichotomous questions formulated as a statement using the yes-no format and 1 multiple-choice questions.

Demographic data including age, gender, clinical experience were also included. Written informed consent was obtained from the participants after explaining the nature and purpose of the study. Data were analyzed by descriptive analysis using percentages from responses to each question.

**Results**

A total of 125 responders participated in the study. Data collected from completely answered 105 proforma were subjected to analysis. The incompletely answered questionnaires (n=20) were excluded from the data [response rate was found to be 84%].

**Demographic characteristics:**

70% of the subjects were female, the most frequent age was 30-49 years (67%) with greater frequency in the working experience of 10 -15 years of dental practice (48%). Most of the respondents (72%) were general practitioners and the remaining 28% were divided between pediatric dentists (8%) and other specialties (20%).

**Knowledge on child abuse**

Results about the level of training and self-education regarding CAN and related items revealed adequacy. 94.28% of the participants agreed on the importance of the role of dentists in diagnosing and reporting child physical abuse cases. 70.47% agreed that craniofacial, head, face, and neck injuries make up more than half of child abuse cases. 87.61% agreed with documentation of CAN in patient record is mandatory. Regarding psychosomatic complaints by the child and physical appearance as poor nutritional status and growth, untidy appearance, untidy clothes a lower proportion of participants were able to correctly identify the signs.
(63.80%, 44.76%, & 11.42% respectively). On the signs (of child abuse 85.71% of the participants were able to recognize appropriately. A larger proportion of dentists (95.23%) knew that reporting physical abuse cases to legal authority is important and 63.80% were aware of the legal procedures involved in reporting of CAN.

**The attitude of dentists on CAN**

The attitudes of the participants are illustrated in Table 2. 36.19% thought that knowledge obtained on child physical abuse at the BDS curriculum was sufficient. 86.66% opinioned that further training is required on how to examine, diagnose, and to report suspected cases of child physical abuse.

**The practice followed by the dentists**

Only 8.57% of the practitioners had suspected physical abuse among patients. A higher proportion of dentists (82.25%) believed that children of employed mothers are more prone to get abused. A very low percentage (5.71%) reported cases of child abuse to Child protective services. Causes of underreporting were fear of legal involvement (25.25%) or lack of response from participants.

**Table 1: KNOWLEDGE QUESTIONS - Knowledge, Attitude, and Practice Regarding Child Abuse and Neglect among Dental Practitioners Rachana**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Questionnaire</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dentists have an important role in diagnosing and reporting child physical abuse cases?</td>
<td>94.28%</td>
</tr>
<tr>
<td>2.</td>
<td>Craniofacial, head, face and neck injuries make up more than half of child abuse cases?</td>
<td>70.47%</td>
</tr>
<tr>
<td>3.</td>
<td>Documentation (signs/symptoms, laboratory evidence, radiographs, photo documentation and videotapes/ audiotapes) of abuse in the patient record is mandatory?</td>
<td>87.61%</td>
</tr>
<tr>
<td>4.</td>
<td>Self-documentation of signs/symptoms of abuse in the patient record is mandatory?</td>
<td>89.52%</td>
</tr>
<tr>
<td>5.</td>
<td>A child psychosomatic complaint is an indicator of child abuse?</td>
<td>63.80%</td>
</tr>
<tr>
<td>6.</td>
<td>Poor nutritional status and growth is an indicator of child abuse?</td>
<td>44.76%</td>
</tr>
<tr>
<td>7.</td>
<td>Untidy appearance, untidy clothes is an indicator of child abuse?</td>
<td>11.42%</td>
</tr>
<tr>
<td>8.</td>
<td>Avoiding eye contact is an indicator of child abuse?</td>
<td>84.76%</td>
</tr>
<tr>
<td>9.</td>
<td>Bite-marks, swollen lips or severely decayed teeth in your patient be related to child abuse?</td>
<td>85.71%</td>
</tr>
<tr>
<td>10.</td>
<td>Ecchymosis, abrasions or lacerations found in an elliptical, horseshoe-shaped or ovoid pattern should be suspected as bite marks?</td>
<td>85.71%</td>
</tr>
<tr>
<td>11.</td>
<td>All bite marks should not be suspected as child abuse?</td>
<td>74.28%</td>
</tr>
<tr>
<td>12.</td>
<td>Unexplained injury, infection (gonorrhoea) or petechiae of the palate may be a result of forced oral sex?</td>
<td>71.42%</td>
</tr>
<tr>
<td>13.</td>
<td>Reporting physical abuse cases to a legal authority is important?</td>
<td>95.23%</td>
</tr>
<tr>
<td>14.</td>
<td>Awareness of reporting a suspicious case of child physical abuse and the legal procedures involved?</td>
<td>63.80%</td>
</tr>
</tbody>
</table>
### Table 2: ATTITUDE QUESTIONS

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Questionnaire</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The amount of knowledge obtained on child physical abuse at your BDS curriculum was sufficient?</td>
<td>36.19%</td>
</tr>
<tr>
<td>2.</td>
<td>Whether further training is required on how to examine, diagnose and to report suspicious cases of child physical abuse (e.g. courses, workshops)?</td>
<td>86.66%</td>
</tr>
</tbody>
</table>

### Table 3: PRACTICE QUESTIONS

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Questionnaire</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>In your clinical practise, have you ever seen a suspicious case of physical abuse among your paediatric patients?</td>
<td>8.57%</td>
</tr>
<tr>
<td>2.</td>
<td>Children of employed mothers are more prone to get abused?</td>
<td>82.25%</td>
</tr>
<tr>
<td>3.</td>
<td>Child abuse is reported more among children studying in rural areas?</td>
<td>59.04%</td>
</tr>
<tr>
<td>4.</td>
<td>Child abuse is seen more among children below the age of 5?</td>
<td>64.76%</td>
</tr>
<tr>
<td>5.</td>
<td>Reported any child abuse cases, if diagnosed?</td>
<td>5.71%</td>
</tr>
</tbody>
</table>

#### 5.1 If yes, to whom did you report?
- a) Child protective service | 66.66%
- b) Law enforcement agency  | 33.33%

#### 5.2 If No, reasons for non-reporting?
- a) Fear of legal involvement | 25.25%
- b) Fear of losing patients and subsequent income | 3.03%
- c) Suspicions are often difficult to confirm | 18.18%
- d) Inadequate training on how to manage child abuse | 21.21%
- e) No response               | 32.32%
Discussion

The present study sought to assess the knowledge, attitudes, and practice of dentists in identifying and reporting cases of child physical abuse as part of an effort to propose and plan future programs and policies for undergraduates. Regarding knowledge about child physical abuse, participants in the present study correctly identified the signs and symptoms of it. Studies have quoted that head, face, and oral cavity are the areas of central focus for physical abuse, due to exposure and accessibility. In accordance, 70.47% of participants in our study were aware that craniofacial, head, face and neck injuries make up more than half of child abuse cases. Similar studies conducted by M. Mogaddam et al. (50%) in Saudi Arabia (73%) and Thomas et al. in the USA (56%, ) revealed that dentists' knowledge of the signs and symptoms of child physical abuse is insufficient in comparison.

In suspected cases of abuse, the actions to be followed by the practitioner were to ask the child and parents about the observed signs, document the signs and symptoms in the child’s file, and check the consistency of the parents’ and/or child’s explanations with the clinical finding. A substantial percentage of participants (87.61%) in our study were aware of the formalities. 76% of the participants in the study by M. Mogaddam et al.6 said that they would document the signs and symptoms and their suspicions in the child’s file.

To suspect a case of child abuse it is essential to have basic knowledge of signs and symptoms and how to diagnose them. The results in this study show that the knowledge of the respondents about the indicators of child abuse was satisfactory except for the untidy appearance and clothes of the child which showed a low percentage of 11.42%.

The majority of respondents knew signs of physical violence in form of bite-marks, swollen lips, ecchymosis, abrasions, or lacerations(85.71%). Awareness of reporting (95.23% ) was also found to be adequate. In another study conducted to assess the knowledge, attitude, and behavior of dentists nearly 76.8% correctly identified the indicators of child abuse.9 As per the response from the participants in our survey, though many of them were able to identify, the need for education on detecting physical signs is realized. It is important to realize that all dental professionals have a unique opportunity and a legal obligation to assist in the struggle against child abuse.2

Attitude

36.19% of dentists stated that the amount of knowledge obtained on child physical abuse at the BDS curriculum was not sufficient and 86.66% felt the need for further training to examine, diagnose and report suspected cases of child physical abuse (e.g. courses, workshops). When professional hygienists were provided post-educational training in identifying and reporting child abuse, post-test questioning indicated that 100% felt they would be able to make a report of child abuse if it was suspected and 96% reported that they knew how to make a report.10 These findings support the importance of courses and workshops to better understand the need to diagnose, document, and builds up the confidence to report cases of child abuse.

Practice:

Dental professionals have an ethical obligation to report suspected child abuse and neglect. Only 8.57% of dentists in our study had suspected physical child abuse cases in their clinical practice and 5.71% of them reported such cases.

The most common feature of emotional neglect is the absence of normal parent-child attachment and a subsequent inability to recognize and respond to an infant’s or child’s needs.2 Similarly participants of our study (82.25%) noticed CAN among children of employed mothers rather than homemakers. 59.04% of the respondents agreed that reports of child abuse is common in children studying in rural areas. In a 30-year follow-up on a prospective investigational study of physical health outcomes in abused and neglected children, it was found that childhood abuse increased the risk for several adult physical and psychosocial problems.10

When asked with the participants about whom to report the suspected child abuse cases, the majority of them answered to report it to the Child Protective Service(66.66%) followed by Law Enforcement Agency(33.3%).
91.43% of dentists didn’t report suspected cases and the greater response was in favor for the fear of legal involvement. Professionals in the field of child protection say that “it is better to over-report than under-report potential abuse”.\(^\text{10}\) Reports should be made to local protective services or law enforcement agencies, or through the National Child Abuse helpline, and should include the name, age, and address of the child, the nature and extent of his or her injury, the person believed to be responsible for the abuse or neglect, and any evidence of previous abuse or neglect. Although child safety protocols are vigilant by the government, this study has identified a definite need to address the subject of ignorance regarding the right authority to report CAN case in dental practitioners.

**Conclusion**

Child abuse and neglect is alarming and since the face and oral cavity are the central focuses of child abuse it is the responsibility of the dentist to acquire knowledge and diagnose such cases. Consequences of abusive behavior toward the child can lead to difficulties in the physical development of the child, health-related issues, psychological signs such as anxiety, depression, insomnia, and fear. Other effects could be a lack of interest in social activities, difficulty in building healthy relationships at work, and home. The magnitude of the problem is larger than estimated. Raising awareness of the warning signs of abuse and educating health care workers, especially dentists, on diagnosis and reporting cases of suspected abuse, would empower them to play an active role in the prevention of child abuse and the protection of vulnerable children.

**Ethical Clearance-** Taken from K.V.G. dental college and hospital ethics committee

(Reference no – IECKVGDC/H/FR07/2019-20)

**Source of Funding-** Self

**Conflict of Interest -** NIL

**References**

Review Article

Indoor Activities for Physical Fitness During Lockdown

Sakshi Manoj Khatri¹, Manoj Patil²

¹ MBBS Intern Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed To Be University). Sawangi (Meghe), Wardha, ²Research Consultant, Jawaharlal Nehru Medical College, Datta Meghe Institute Of Medical Sciences (Deemed To Be University). Sawangi (Meghe), Wardha

Abstract

The on-going novel corona virus 2019 from the outbreak of china is affecting many more countries almost 213 have been reported. The highest number of cases till 10th of July 2020 is from the country united states. India is the 3rd most country which has been affected by novel corona virus 2019. The least country which is affected and recovered also is an islands of saint pierce and Miquelon. So for the measure of preventing the novel coronavirus the most of the government has declared complete lockdown especially in India there were complete lockdown from 23rd march 2020 and this whole pandemic thing has affected a lot of people, students, children’s, elders and all as all gyms and garden were closed people were not able to go out so then what about their fitness in this pandemic. New data has been come out that while doing exercise may reduce the risk of breathlessness like respiratory distress syndrome, which is one of the major causes of death with coronavirus 19. (covid19). Breathing disorder, a severe lung condition. Physical activity should not be confused with exercise. Physical activity means movement of body that uses energy. It can be moderate or vigorous intensity. Let’s be active everyone and every day. Regular physical activity reduces the risk of non-communicable diseases like blood pressure, ischemic heart disease, stroke, blood sugar level, colon and breast cancer. All conditions that increase the susceptibility to covid-19. And mainly in the lockdown the new things for people it will help you from to maintain your weight and from anxiety and depression.

Key words: Fitness and health come first proper diet and proper care will not lead to coved.

Introduction

At the beginning of lockdown people were enjoying the new change unhealthy lifestyle with a lots of home food and zero exercises. All progress take place outside the comfort zone. As we know good things come to people who sweats. Once you see the result it will make yourself happy and feel good. As health care provide, we need to considered that what can we do for the physical fitness by staying indoors and doing activities. From building up for proper routine to right from waking up till night. Rewarding yourself on reaching a milestone, giving proper time for exercise. Adopting various method to not get obsolete. In the form of running, walk at home, programs, weight training accompanied with proper diet, sleep and water intake.

Healthy health at home. This pandemic is not easy for anyone especially people who work out from the home because sitting at home doing the work without any movement may affect both physical and mental status of the person. But the time like this it’s very important for all ages of people to do activities at this point of time. By doing these 3 to 5 mins also it will not only ease your muscles but also blood circulations also.

It will affect both body and mind. Not only these it will also affect urn bone and muscle strength, flexibility and fitness.

It’s even harder for the people who don’t do all this activity but for time being its very much essential to do.

Indoor activities for physical fitness during lockdown

Ten boons of physical activities:

It will reduce your health risk. Change what you eat and when you eat. Stop smoking and control your blood pressure by eating healthy. So that way it’s helping your blood vessels to get clear. Which helps your heart strong and diseases free? As I already said stop smoking to
strengthens your lungs as smoking impairs lung function making it harder for the body to fight off coronaviruses and other diseases. Reducing the risk of diabetes mellitus as the patients who had diabetes more likely to have serious complications and patient may lead to death. Weight management not only for the fear of coronavirus but also for your own fitness as you know “health is wealth”. (1) So, weight management should be seen in the fitness list. It makes your bone strong and it also helps in preventing the cancer like colon and breast and many more. It regulates the hypertension the main reason is atherosclerosis. It also improves your enthusiasm level and it also builds up your stamina. And lastly and most importantly it enhances emotional wellbeing. And control in your delirium, anxiety and depression. (2)

**Getting started**

Fitness studio shutting and we are adjusting the new life. You are your own gym. To make health habitual in this pandemic we need to Thake this as an opportunity to promote the healthy lifestyle to prevent the diseases and for our mental health. Making new healthy habit. Deciding on a goal to achieve a new healthy habit. Plan when where and what will you be doing. (3) It will get easier with time and in about ten weeks you will find out that automatically you’re doing without any great initiations but you will feel good to do it and it will be a congratulations to you for making the healthy habit. Create a goal to have more wears and green vegetables not only that but to also have more proteins also. Not only this normal fluid intake in man per day is 3.7 liters and women is 2.7 liters.it will help you to lose your weight it will also boost your metabolic rate also. (4) It will also help you to relive the headache, constipation and kidney stones etc.

**Home fitness revolution**

As the covid19 is affecting people. As people are not going out due to the mode of transmission it’s better to have social distancing and take as much as precautions we can take. As government announces the complete lockdown in India and it includes all thing gymnasiums, pools, Zumba classes, dance classes and garden places also where people use to go every day for their fitness but now what as the things are being closed so here we come with the unique and the useful thing like home fitness mutiny. (5) In esteem to these issues, gyms and fitness centers like dance classes, yoga places are throughout in the India have been closed during the period to ensure the shielding of its citizens. (6) Before considering this covered 19 the people has actually had the apps for indoor cycling like Zwift, indoor cycling, cycle go indoor, full gas and many more. Hence know people are considering this more as there would be the new trend going in with social distancing norm of being self-distanced home workout. (7) Workout in simple language is it effective to do workout at home in short and simple = yes why not? You can complete a perfectly good fitness routine at home using nothing more than your body weight. (8)

*Triceps dips=* it’s one of the top most great and effective body weight exercise that not only builds but strengthen your arms and shoulders. It can be done anywhere and its simple to do.it is for upper body strengthen workout. Yeah, the muscles part which work for the triceps dips are anterior deltoid, the pectoralis muscles like sternal, clavicular and minor. And the rhomboid muscle of the back. (9)

**How to do triceps dips?**

1) while you use your hand grip the front edge of anything like chair, stool, table or stairs also you can use.

2) remain in one place in air with your buttocks region just off and make sure your feet flat, to make the bending of the legs so that the thighs are parallel to your floor where ever you are doing exercise. (10)

3) the starting position is to straighten your arm. It will help you to do triceps dips more evenly and flexibly.

4) bring your arm to 90 degrees make sure it is in comfortable position.

5) engaging your triceps and bringing back to first.

That’s one act!

Simultaneously do inhalation and exhalation also. Don’t do sinking of shoulders. There should be action of shoulder joint flexion and extension that would be engaging the triceps muscles. (11)
Figure 1: Pot rating how to do triceps dips.

_Disk exercise:_ - Plank don’t just work your core they work for your entire body. Plank is one of the most and the best calorie burning and beneficial exercises. A plank hold engages multiple muscle at once there by benefiting the core strength of your body. (12) Not just burning the fat around your abdomen area they also work by giving you good posture and flexibility. (13) The 2 vital components it will help you to keep your shoulder back and lower back in neutral position while sitting or standing. (14)

![Plank exercise instructions](image)

**THE 15-MINUTE PLANK WORKOUT**

Try this routine two or three days a week. Perform 10 to 15 reps of each exercise, moving from one to the next without resting. Rest for one minute, then repeat the circuit up to four times total.

1. **PLANK ROW AND KICKBACK**
   - Complete all reps, then switch sides

2. **PLANK WITH FRONT RAISE**
   - Complete all reps, then switch sides

3. **ROTATING T EXTENSION**

4. **MOBILITY EXTENSION**

![Plank exercise instructions](image)

Figure 2: How to do plank? (15)
Yoga and mental health

Yoga has all three things for health that are physical, mental and spiritual from prehistoric India. Freedom and flexibility to get on and do. It also helps in various form like to cure from depression, fatigue, stress and many more. Not only all this the positive vibes that people needed in this pandemic the yoga has that efficacy to do. It removes toxins from our body. Physically mentally fit is both important in today’s time as in this pandemic is something new for people.

There are some types of yoga you should do it to keep your mental health good. It will help you to have satisfaction.

1) Yoga Sana: - standing, sitting, prone and supine lying. Do it with breath awareness. Cardiac patient shall do it with care. It helps to improve chest expansion and cardio pulmonary functions.

Surya namaskar (the ultimate asana)

Strengthen your back as well as your muscles and bring down blood sugar level. Helps to improve metabolism.

Figure 3: Types of soya namaskar asana.

There are 12 asanas in this soya namaskar Ana the first one is namaskar asana. Second one is hastotanasana where hand stretching is the pose. thirdly it is podahastosana this is your feet to hand pose. Then fourthly you have ashwa sanchalanasana this is horse parading pose. Then come 4-point stick pose which is also called as chaturanga dandasana. Sixthly, it is downward facing dog pose adhomukha shewanasana. Then there is 8-point namasker which is also known as astanga namaskara. Bhujagasana that is cobra pose it’s the 8th position. In 9th position it is like baby pose also called as balasana. 10th position is horse parading pose which is ashwa sanchalanasana and second lastly there is foot to hand asana which is padahastasana and lastly your 12th position is hand stretching pose that is hastottanasana.

1) namaskarasana. Benefits= mental health is affecting this pandemic so this asana helps your calmness of mind and breathing regulation.

2) hastotanasana (hand stretching pose).

Benefits= infusing of the blood in Cerro-spinal column and it is helping on stretching of posture.

3) podahastosana (feet to hand pose).

Benefits= this asana helps you to reduce your adipose fat which also encourage you for the weight loss. As there is infusing of the blood in the cerebro-spinal system so the blood is also going in to abdominal organs which helps in functioning and healthy system of lifestyle.

4) ashwa sanchalanasana (horse parading pose).

Benefits= by this asana you’re getting strength and flexibility to your hip region, pelvic are and muscles and shoulders. Strengthening is benefited for your musculoskeletal system.

5) chaturanga dandasana (4- point stick pose).

Benefits= this asana helps you to maintain your straight and stiff posture of the back and abdominal muscles. It also helps in reduction of muscles by holding of your abdomen muscles like transverse abdominis and rectus abdominis.

6) adhomukha shewanasana (downward facing dog pose).

Benefits= this asana helps in flexibility, strengthen for your legs muscles sartorius, gastrocnemius muscle like and your thigh muscles like semimembranosus muscle, semitendinosus muscle, gracilize muscle.
7) astanga namaskara (8-point namasker).
Benefit= it’s an interim asana.
8) bhujangasana (cobra pose).
Benefit= it’s helps in stretching.
9) balasana (baby pose).
Benefit= it helps you to reduce your neck and back pain, simultaneously, it helps you to reduce your stress and fatigability and off course your weight.
10) ashwa sanchalanasana (horse parading pose).
Benefit= it is good for musculoskeleton system.
11) padahastasana (foot to hand asana).
Benefit= stretching and strengthen of the muscles.
12) hastottanasana (hand stretching pose).
Benefit= for calming of the mind.

There are some contraindications in which you should avoid surya namaskara (the ultimate asana) like the person who has went in recent surgery or who has severe back ache or vertigo don’t do it stop immediately.

Meditation

Meditation is like giving a hug to ourselves, getting in touch with that awesome reality in us. While meditating we feel a deep sense of intimacy of god, a love that is inexplicable. Meditation is a process of slowing down yourself, calming down yourself, don’t worry, don’t hurry…trust the process. Your goal is not to battle with mind, but to witness the mind. Not only for attention and awareness but also for mentally calm emotional state. It’s a part of self-realization and enlightens. meditation is under the effect of psychological, neurological and cardiovascular and many more other affects. Meditation helps in improvement in anxiety and depression and other effects also. The anxiety and depression are affecting more people in this pandemic of India. Mediation also help in irritable bowel syndrome(is), insomnia and post-traumatic stress disorder.

Why exercise?

· Improves the quality of life.
· It will help you in sleep.
· Reduces stress.
· Improve asthma control.
· You will happy.
· It will help your skin health.
· Relaxation.
· Builds aerobic power.
· Reduces the risk of arthritis.
· Maintain the immune system.
· Improves your mood.
· Lower dementia risk.
· Boost memory.
· It helps to not develop obesity.

This all we need in this pandemic to a better life and lifestyle.

Exercising while social distancing

As the time passing there are going to be unlocking to all the system as life don’t stop. As people will come out keeping in mind covid 19 is there wearing of mask and self-distancing is must. Getting some fresh air without coming in contact with people. You can do walking, cycling and jogging and all. Use the mask which is washable as there is allergy season going on. You should exercise alone. Not at all in the groups it
Exercise after covid19

Physical fitness is must in today’s time. Not only for just to be fit also for your mental health. It reduces so many things just buy doing exercise the non-communicable diseases like hypertension, diabetes, colon and breast cancer and many more. It makes you feel, good, happy, energetic. So why not to continue the exercise and say no to the diseases? Is it too much to ask for? For those who are doing it initially knows they are on the right track. Those who started doing in this pandemic here you go with starting game of your good health. The one thing in this pandemic you can say that health is your friend and you get happiness while doing so let’s make a promise to our self-healthy lifestyle say no to diseases. It’s for our selfcare. For beater future and healthy life style. Do it for today and for coming tomorrows. One last thing maintains a persistence, consistency and discipline.

From here where?

As we know food is a great friend of our human being. It has help in this pandemic also for many people but it had led to the cause also like obesity. Food in some amount is good not being overeaten. Weight gain it can encourage many people to start a new lifestyle a lifestyle of being fit and fresh mind and being not stressful. Being responsible and dutiful like sun, stay positive and being fit. Search a beauty in your lifestyle by doping exercise, work out. Or anything you called. Strive for progress not for perfection in your fitness. Physical fitness or exercise is not only one of the most important keys to a healthy body, it’s the basis of dynamics and creative intellectual activity. And remember the last thing the famous quote “health is wealth”. Take care of your body it’s the only place you have to live. Put the excuses aside.

Conclusion

Lockdown implemented in an attempt to contain the COVID 19 virus which is unpredictable for the countries and representing the major societal changes for people’s with mental health and physical health. Any health care professional should use this time as window opportunity to provide physical health and counselling to their patients. Healthy eating doing exercise sleeping on time making your own health good. Will conclude to “HEALTH IS WEALTH”.

Conflict of Interest: None

Ethical Approval: IEC , DMIMS, Wardha

Funding: DMIMS, Wardha.

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Knowledge and Preventive Measures Adopted by Allied Health Care Professional Students to Control Transmission of COVID-19 Pandemic Infection During Lockdown

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Abstract

Introduction: After declaration of COVID-19 infection as pandemic by World Health Organisation (WHO), various preventive strategies have been intended to mitigate disease spread and control the infection rate such as isolation of patients, social distancing, hygienic practices; covering mouth and nose, restriction of mass gathering in society. Hence we planned this survey to assess the knowledge and preventive measures adopted by students to control COVID-19 infection during lockdown.

Methods: This cross sectional study was conducted among students of School of Allied Health Sciences, DMIMS, Wardha during April-May 2020. First year was selected randomly and all 168 students were approached by internet for sharing questionnaire after IEC approval. Received responses is analysed in the form of descriptive statistics.

Results: Total 139 students responded. 38.85% students understood the relationship between mass gathering and transmission of COVID-19 infection at community level. Only 34(24.46%) students were aware about safe social distance. Less than half of the students 66(47.48%) were aware about minimum 20 seconds needed to hand-rub by the alcohol based sanitizer. About 108(77.7%) students maintained the social distancing sincerely, only 74(52.5%) students always used to wear the mask and; 100(71.94%) participants always used to wash their hands.

Conclusions: Though some of the students adopted standards practices, others observed to be considering it as less important. Such survey can be conducted among in various institutions to assess the awareness and shortfall in practices. Awareness level can be raised through dedicated online awareness program.

Key words: Allied Health Sciences, COVID-19, Lockdown, Pandemic, Preventive measures, Students

Introduction

COVID-19 infection was declared as pandemic by the World Health Organisation (WHO) on 11th March 2020.[¹,²] Thereafter, it changes the world scenario and life of human being. In the present century, this infection causes global crisis for human species.[³]

Main source of this infection is patients with COVID-19 and most common routes of transmission are droplets and close contact. Patients are recovering by providing symptomatic and palliative care.[⁴,⁵] As no specific vaccines and treatment is available till date, only implementing and adopting preventive measures at individual and community level is the golden rule
to reduce the morbidity and mortality by COVID-19. Various preventive strategies are isolation of patients, social distancing, hygienic practices; covering mouth and nose, restriction of movements and mass gathering in society.\textsuperscript{[6,7]}

As virus is highly infectious; to date, COVID-19 has affected over 5,701,337 people worldwide, resulting in over 3,57,688 reported deaths.\textsuperscript{[8]} As of 1\textsuperscript{st} June 2020, confirmed cases of corona virus infection were noted as 1,93,473 in India; out of these 94,963 were active cases. Though 93,062 patients recovered, there were 5,437 deaths in the country.\textsuperscript{[9]} Mass gathering leads to close contacts of people and maintaining the social distance is the first one step to curtail the spreading of infection.\textsuperscript{[10]} Wearing of mask is the second essential action, if person has to go in society for the unavoidable task.\textsuperscript{[11]} As the virus may get inculcated, if infected hands come in contact with mucosal surface such as eyes, nose and mouth, so the importance of hand hygiene can’t be neglected.\textsuperscript{[12]} There is need to assess the preventive measures adopted in the community to ensure the control of spread of infection safety.

**Objectives:** To assess the knowledge and preventive measures adopted by Allied Health Care professional students to control the transmission of COVID-19 infection during the period of lockdown.

**Material and Methods**

This cross sectional study was conducted in School of Allied Health Science, DMIMS during April-May 2020. One of the graduation years i.e. first year was selected randomly and all 168 students were approached by internet. Questionnaire was prepared by referring guidelines from World Health Organization (WHO)\textsuperscript{[13,14,15]} and Ministry of Health and Family welfare, Government of India.\textsuperscript{[16,17]} Questionnaire was shared among students through internet after taking their informed consent. Received responses is analysed in the form of descriptive statistics frequency and percentage.

**Results**

Out of 168 students, 139 had responded to the shared questionnaire. Out of total responded, male participants were 60(43%) and 79(57%) were female students and; most of the students (33.09%) were of 18 to 19 years. Maximum participants i.e. 100(72.50%) students were residing in the green zone. Social media was observed to be the commonest source of information for 109(78.42%) students.[Table I]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n=139)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-18</td>
<td>36</td>
<td>25.9</td>
</tr>
<tr>
<td>18-19</td>
<td>46</td>
<td>33.09</td>
</tr>
<tr>
<td>19-20</td>
<td>30</td>
<td>21.58</td>
</tr>
<tr>
<td>20-21</td>
<td>15</td>
<td>10.79</td>
</tr>
<tr>
<td>&gt;22 &amp; above</td>
<td>12</td>
<td>8.63</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>43</td>
</tr>
<tr>
<td>Female</td>
<td>79</td>
<td>57</td>
</tr>
<tr>
<td><strong>Residential area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>49</td>
<td>34.5</td>
</tr>
<tr>
<td>Urban</td>
<td>90</td>
<td>65.7</td>
</tr>
<tr>
<td><strong>City or town declared as risk zone of COVID-19 by Government</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>23</td>
<td>15.9</td>
</tr>
<tr>
<td>Orange</td>
<td>12</td>
<td>8.7</td>
</tr>
<tr>
<td>Green</td>
<td>100</td>
<td>72.5</td>
</tr>
<tr>
<td>Nothing</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Source of information*</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social Media</td>
<td>109</td>
<td>78.42</td>
</tr>
<tr>
<td>Friends</td>
<td>42</td>
<td>30.22</td>
</tr>
<tr>
<td>Health staff</td>
<td>44</td>
<td>31.65</td>
</tr>
<tr>
<td>Family members</td>
<td>52</td>
<td>37.41</td>
</tr>
<tr>
<td>Other</td>
<td>32</td>
<td>23.02</td>
</tr>
</tbody>
</table>
In the present study we assessed the knowledge regarding the spread, preventive and control measures of COVID-19 infection. 91(65.47%) participants responded for coughing/sneezing, followed by 70(50.36%) participants responded for close contacts as a mode of spread of COVID-19 infection in the community. About similar pattern of distribution of responses was noted for route of transmission in a human i.e. 85(61.15%) for coughing/sneezing and for close contacts 71(51.08%).

Most of the students i.e. 104(74.82%) were found to be aware that most essential preventive measures can be taken by general public is social distancing. However, only 81(58.27%) of students believed that social distancing should be adopted by everybody as a preventive measure.

According to 23(16.55%) of the students, hand hygiene and; 27(19.42%) students, use of mask is the most essential measures that should be adopted by everybody.[Table II]
Table III: Knowledge about Covid-19 virus among the Allied Health Science students

<table>
<thead>
<tr>
<th>Social distance to prevent COVID-19 inf.</th>
<th>Number (%)</th>
<th>minimal time for alcohol-based hand rub to kill Cov-19 virus</th>
<th>Number (%)</th>
<th>Effective vaccine Available</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 foot</td>
<td>37 (26.62)</td>
<td>5 sec</td>
<td>18 (12.95)</td>
<td>Yes,</td>
<td>9 (6.47%)</td>
</tr>
<tr>
<td>2 feet</td>
<td>15 (10.79)</td>
<td>10 sec</td>
<td>39 (28.06)</td>
<td>No,</td>
<td>58 (41.73)</td>
</tr>
<tr>
<td>3 feet</td>
<td>34 (24.46)</td>
<td>20 sec</td>
<td>66 (47.48)</td>
<td>Don’t know</td>
<td>72 (51.80)</td>
</tr>
<tr>
<td>4 feet</td>
<td>13 (9.35)</td>
<td>30 sec</td>
<td>13 (9.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4 feet</td>
<td>41 (29.50)</td>
<td>&gt;30 sec</td>
<td>3 (2.16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We also tried to uncover the basic and common knowledge about the corona virus among the participants. It is noted that, only 34(24.46%) students were aware about 3 feet (one meter) social distance should be maintained to prevent COVID-19 infection. Most of the students i.e. 41(29.50%) believed that social distance of more than four feet would be safe. Whereas, 37(26.62%) and 15(10.79%) students pointed for 1 foot and 2 feet as a safe social distance in these COVID pandemic. Minimal time take for alcohol-based hand rub to kill Cov-19 virus is 20 seconds and same time for soap rubbing.[14] Only 66(47.48%) responded this theme correctly, other 18(12.95%) and 39(28.06%) students ticked the 5 and 10 seconds required time to kill the virus respectively. 16(11.51%) students choose safer side for the same question i.e. time require to disinfect hands is more than 20 seconds.[Table III]

Table IV: Practices adopted by Allied students to prevent COVID-19 infection

<table>
<thead>
<tr>
<th>Practices</th>
<th>Always (%)</th>
<th>Most of the time (%)</th>
<th>Sometimes (%)</th>
<th>Rarely (%)</th>
<th>Never (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social distancing</td>
<td>108 (77.7)</td>
<td>24 (17.3)</td>
<td>4 (2.9)</td>
<td>0 (0)</td>
<td>3 (2.2)</td>
</tr>
<tr>
<td>Wearing mask for outdoor</td>
<td>74 (52.5)</td>
<td>45 (32.4)</td>
<td>6 (4.3)</td>
<td>10 (7.2)</td>
<td>5 (3.6)</td>
</tr>
<tr>
<td>Washing hands after returning from outside</td>
<td>100 (71.94)</td>
<td>26 (18.70)</td>
<td>5 (3.6)</td>
<td>6 (4.32)</td>
<td>2 (1.44)</td>
</tr>
<tr>
<td>Wear eye protection for outdoor</td>
<td>66 (47.48)</td>
<td>44 (31.65)</td>
<td>17 (12.23)</td>
<td>4 (2.88)</td>
<td>8 (5.75)</td>
</tr>
</tbody>
</table>
In the present study, 108 (77.7%) students maintained the social distancing always and 24 (17.3%) students maintained it most of the time. Only 74 (52.5%) students always used to wear the mask for outdoor activity and 45 (32.4%) students followed this practice most of the time. After returning from outside at home, 100 (71.94%) participants always used to wash their hands and 26 (18.70%) students followed this practice most of the time. [Table IV]

![Methods of disposed off used mask by the students during COVID-19 pandemic](image)

About 48.92% students followed the practice of throwing the mask in dustbin and 27.34% students used to wash the mask for reuse. [Fig-2]

**Table V: Frequency and Purpose of going outside during Lockdown Period**

<table>
<thead>
<tr>
<th>Frequency of moved out from home</th>
<th>Number (%)</th>
<th>Purpose of going outside from home*</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>14 (10.1)</td>
<td>Purchasing vegetables</td>
<td>65 (46.76)</td>
</tr>
<tr>
<td>Alternate day</td>
<td>4 (2.9)</td>
<td>Purchasing groceries/ stationery/milk/fruit</td>
<td>38 (27.34)</td>
</tr>
<tr>
<td>Twice /thrice a week</td>
<td>7 (5)</td>
<td>Meeting people (friends, neighbours, relatives)</td>
<td>2 (1.44)</td>
</tr>
<tr>
<td>Once in a week</td>
<td>27 (19.4)</td>
<td>Office work</td>
<td>5 (3.6)</td>
</tr>
<tr>
<td>Occasionally</td>
<td>41 (29.5)</td>
<td>Exercise</td>
<td>13 (9.7)</td>
</tr>
<tr>
<td>Never</td>
<td>46 (33.1)</td>
<td>Not applicable</td>
<td>46 (33.1)</td>
</tr>
</tbody>
</table>

(*multiple response allowed)
When we asked the participants about frequency of going outside, it is noted that one third of them i.e. 46(33.1%) follows the rules of lockdown sincerely by remaining at the home. 41(29.5%) students moved out of home occasionally, whereas 27(19.4%) once in a week and only 14 (10.1%) students moved out daily. Common purpose of going outside was observed for purchasing vegetables/groceries/stationary/milk/fruits by 65(46.76%) and 38(27.34%) the students respectively. [Table V]

Discussion

We have received responses from almost all the participants in first half of the May 2020. Type of risk zone for Novel corona virus is observed to be very dynamic; it changes anytime during the week. Wardha district was declared green zone up to the 10th May 2020, where most of the students are residing during the lockdown. This may be the reason for maximum students responded for green zone during the data collection period. Social Media is playing the major role in making the community aware about the status of COVID-19.

Mass gathering is prohibited to avoid community transmission. This should be clear among the people. Only 38.85% students of Allied health sciences understood the relationship between mass gathering and transmission of infection at community level. It is equally important for a healthy individual to protect from droplets in the air by covering nose and mouth and, for the patient by adopting cough etiquettes. In present study, 61.15% participants responded correctly for coughing and sneezing main route of COVID-19 transmission at individual level. There is need to improve the specific awareness regarding mode of transmission among the students.

Social distancing is core strategy during this lockdown period. Important advice for general public by World Health Organization is to maintain the social distance at least three feet and so everybody should be aware of and follow it sincerely. [6,9] About three fourth (74.82%) of the participants believed that most essential preventive measures can be taken by general public is social distancing. However it is equally important to understand that everybody is involved in general public and it is the responsibility of each and every person to maintain the said social distance as a preventive measure. Students were observed to be not sure about personal level responsibility of social distancing and hence only 58.27% students could mark it correctly.

Less than half of the students (47%) were aware about the particular time needed to hand rub by the alcohol based sanitizer i.e. 20 seconds. Though very few students (11%) had chosen safer time i.e. 30 or >30 seconds for hand rub, still near about half of the students were unaware of it and might be practicing unsafe hand hygiene. Such unsafe practices may increase the chances of getting corona virus infection to an individual and community further. No specific vaccine is available for the COVID-19 infection till date. Most of the students were unaware the exact status of vaccine availability, so basic knowledge about COVID-19 disease needs to improve among students.

Various types of information are accessible on social media. However, not all students were accessing this information. Students have common tendency of following academic instructions sincerely. This may be the reason that, few students remain ignored from some of the facts published or shared through social media. In such situation, institution can plan the online session for these students to increase the particular knowledge.

Most of the students responded that, they were maintaining the social distancing. However, first of all one should know the standard or required distance to be maintained to avoid the catch of corona virus infection. As per the guidelines, minimum 3 feet social distance is to be maintained for prevention of corona virus infection. [10,18,19] More than 3 feet distance is safer, but less than 3 feet distance promotes the transmission of corona virus infection from person to person. In our study, 52(37.41%) were observed to be unaware of minimum social distance to be maintained in the society.

Hand hygiene and use of mask plays vital role in preventing of getting infection. As per WHO, wearing of mask is compulsory for the COVID-19 positive patient to control the diseases spread. [20] But it is equally important to protect our self from getting infection. One of the measures for it is wearing the mask by healthy or apparently healthy individual to cover nose and mouth. [21] In the present study only half of the participants were sincerely using mask every time for outdoor activity and about one third (32.4%) were trying to keep up
this practice by following it most of the time. But other students were taking it casually by allowing themselves going outside without covering their nose and mouth. This practice may be harmful for the individual and so for the community. However, students were following safe practice of mask disposal.

WHO recommended frequent hand washing not only at the home but also in outdoor settings.[22] However, about two-third of studied participants were following the practice of hand hygiene at home sincerely. Some of the students moved out during this lockdown and reason for outing was very rational i.e. for obtaining daily needs.

**Conclusion:** During this pandemic of COVID-19, it is important to follow some of the practices sincerely and always rather than missing any time. Students are needed to be aware of standard guidelines along with motivate the participants for adoption of safe practices. Our team has shared the important health awareness information which is published by WHO as an ‘Advice for Public’ among the students of Allied Health sciences.

Research in this area is observed very rare during literature search. It is recommended that, such survey can be conducted among the students of various institutions to assess the awareness and shortfall in practices adopted by them for prevention of COVID-19. So that gap in the knowledge, understanding and practices can be recognised and corrected further through academic learning session. Nowadays, teaching institutions are closed, still online academic teaching is going on. So, we can grab this opportunity to raise the awareness level among the students through dedicated online awareness program.

**Ethical Clearance:** Done

**Funding:** None

**Conflict of Interest:** None

**References**


9. covid19india.org: Coronavirus Outbreak in India https://www.covid19india.org/


To Assess the Knowledge Regarding HIV/AIDS among New Entrant of the Nursing Institute in Wardha District

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Abstract

Introduction: One of the world’s most significant public health issues has been HIV infections and AIDS (HIV / AIDS). The 1 percent of deaths worldwide are responsible for HIV / AIDS Knowledge of HIV / AIDS among students is crucial to the elimination of disease propagation. Besides, young adult university students may start their own families someday and some may be health care providers in the future. Data about Knowledge, regarding HIV/AIDS are needed to sensitize and create awareness among nursing students as they were becoming health care administrators and providers in the future. Aim: To determine the knowledge regarding HIV/AIDS among new entrants of the nursing institute. Materials and Methods: A cross-sectional study was carried out among new entrants of the nursing institute in Wardha district. The students were chosen randomly. This cross-sectional descriptive research was carried out by pre-designed, pre-tested, and semi-structured surveys of eligible 296 nursing students in their first year of life. First-year Basic Bsc nursing100 students, GNM100 students, ANM 96students in the age-group of 17-19 years on 1st, 4th, and 7th Oct’ 2016, respectively. After the study purpose was clarified, the students received 45 minutes without shared consultation under the supervision of the investigator, to obtain informed verbal consent and maintain confidentiality. Students have been given the right to withdraw without fear or obligation from the study at any time during their data collection, but none declined to take part. The research variables included demographic variables such as sex, professional education, the difference between HIV / AIDS, routes of transmission, prevention strategies, category of behavioral risk, and antiretroviral therapy, etc. Result: Out of 296 total students, 123 (51.9%) were women and 103 (44.0%) were rural native students. The mean knowledge score for the professional education of ANM was 17.35± 3.59, for GNM it was 13.00±3.61and for B.Sc. Nursing it was 17.37±4.31. The majority of the lot was aware of all four routes of infection transmission and methods of prevention. When the mean data score for the three new entrants is compared, no major statistical difference except ANM (P=.999) is noted.

Keywords: Evaluate, Awareness, Human immunodeficiency virus infection, Fresh candidate, Nursing Institution

Introduction

By 2030, the world has vowed to end the AIDS epidemic. Significant obstacles have yet to be met. The world’s number of new HIV infections, which amounted to 36.7 million (34.0 to 39.8 million), stood at about 2.1 million [1.8 million – 2.4 million] in 2015.¹

The latest HIV figures for India for 2015 are projected at 0.26% (0.22%–0.32%) by 2015 to include the number of adults (15–49 years), in India. In 2015, the prevalence of adult HIV among men is estimated at 0.30% and among women at 0.22%. In 2015, the most reported adult HIV prevalence of Manipur in the United States was 1.15%, followed by Mizoram (0.80%), Nagaland (0.78%), Andhra Pradesh & Telangana

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India is the third-largest HIV outbreak in the world. In 2017, an additional 0.2% of adults were between 15 and 49 years of age. This number is small relative to most other middle-income nations, but 2.1 million are HIV sufferers due to the large population of India (1.3 billion people).

As most people in India are considered infection-free, education in information is one of those key policy items. Therefore, knowledge training will continue to regularly assess the level of awareness about HIV / AIDS to provide policymakers with insights on how education can be better improved. A survey was carried out in this context to assess the extent of the awareness of HIV / AIDS among first-year professional university students in India for the admission year 2016.

Materials and Methods: A descriptive cross-sectional study was conducted from 10th Oct 2016 to 15th Oct 2016 and the setting was selected under the ambit of Datta Meghe Institute of medical sciences, Smt. Radhikabai Meghe Memorial College of Nursing and Florence Nightingale College of Nursing in Sawangi Meghe Wardha after getting ethical permission (Ref. no: DMIMS(DU)/IEC/2016-17/6061). By using the purposive sampling technique, 296 students of new nursing entrants of all three courses of nursing i.e. Auxiliary nurse midwifery course duration two years, General nursing and midwifery three years, and four years degree course Basic B.Sc. Nursing. The nursing students were informed and explained the objective of the study. The written informed consent dully signed individually by them was obtained. The inclusion criteria were: students who are willing to participate in the study and senior students were excluded from the study. Demographic variables were collected in terms of Age, Gender, Stream of Education in 12th standard, Stream of professional education, a previous source of information about HIV/AIDS, and Family members in the medical profession.

A semi-structured questionnaire has 30 multiple choice questions and these were classified in different areas, such as (i) the meaning of HIV/AIDS and its mode of transmission (ii) Medical management and prevention. The questionnaire was prepared based on the extensive review of the literature and clinical experiences of handling HIV patients. Each correct answer carries one mark and the total score is 30. The prepared tool was validated by ten experts, out of the eight were from the nursing department, one was from the Department of medicine and one was from the physiology department. Reliability analysis was done by Guttman split-half coefficient and was 0.90, hence the tool found reliable, valid, and feasible. The interview technique was processed for 296 samples was planned to gather demographic information and the knowledge on HIV/AIDS including the meaning of HIV/AIDS and its mode of transmission, medical management, and prevention questionnaire was administered, each sample requires meantime 30 minutes to complete the questionnaire. As collected, the responses were arranged in tabular form to conduct statistical analyzes which are mentioned in the following sections.

Statistical Analysis

The collected data were coded, tabulated, and analyzed by using descriptive statistics (mean percentage, standard deviation) and inferential statistics. Association of knowledge with demographic variables was done by one way ANOVA test and independent t-test and for the multiple comparison Tukey test has been used.
## Results

### Table 1: Distribution of subjects according to their demographic characteristics

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>No. of postgraduate health care professionals</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-22 yrs</td>
<td>263</td>
<td>88.85</td>
</tr>
<tr>
<td>23-27 yrs</td>
<td>27</td>
<td>09.12</td>
</tr>
<tr>
<td>28 to 30 yrs</td>
<td>06</td>
<td>2.03</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>13.85</td>
</tr>
<tr>
<td>Female</td>
<td>255</td>
<td>86.15</td>
</tr>
<tr>
<td>Stream of professional education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANM</td>
<td>98</td>
<td>33.11</td>
</tr>
<tr>
<td>GNM</td>
<td>100</td>
<td>33.78</td>
</tr>
<tr>
<td>B.Sc.</td>
<td>98</td>
<td>33.11</td>
</tr>
<tr>
<td>Previous source of information about HIV/AIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>176</td>
<td>59.46</td>
</tr>
<tr>
<td>No</td>
<td>120</td>
<td>40.54</td>
</tr>
<tr>
<td>If your classmate got infected with HIV/AIDS, would you continue interacting with him/her?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>234</td>
<td>79.05</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>20.95</td>
</tr>
<tr>
<td>Family members in the medical profession</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94</td>
<td>31.76</td>
</tr>
<tr>
<td>No</td>
<td>202</td>
<td>68.24</td>
</tr>
</tbody>
</table>
Table 1. Shows that up to 88.85% students age was 18–22 years, 09.12% were 23–27 years of age and 28–30 years of age just 2.03%, Up to 86.15% female, and 13.85% male students, as per stream of professional education 33.78% GNM, 33.11% and ANM and BSc Nursing respectively. HIV / AIDS is well known to most students, e59.46%, Most of the students is 79.05 % able to communicate with their students who have HIV / AIDS infected, The majority of family members of students 68.24 % are in the medical profession.

Table 2: knowledge score of regarding HIV/AIDS among new entrant of nursing institute

<table>
<thead>
<tr>
<th>Level of knowledge score</th>
<th>Score</th>
<th>Percentage score</th>
<th>Knowledge score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>Poor</td>
<td>1-6</td>
<td>0-20%</td>
<td>02</td>
</tr>
<tr>
<td>Average</td>
<td>7-12</td>
<td>21-40%</td>
<td>72</td>
</tr>
<tr>
<td>Good</td>
<td>13-18</td>
<td>41-60%</td>
<td>141</td>
</tr>
<tr>
<td>Very Good</td>
<td>19-24</td>
<td>61-80%</td>
<td>77</td>
</tr>
<tr>
<td>Excellent</td>
<td>25-30</td>
<td>81-100%</td>
<td>04</td>
</tr>
<tr>
<td>Minimum score</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Maximum score</td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Mean score</td>
<td></td>
<td></td>
<td>15.89 ±0.253</td>
</tr>
</tbody>
</table>

Table 2. Shows that (0.68%) had poor knowledge, (24.32%) average knowledge and (47.63%) were good, (26.01%) were very good and (4%) were excellent. The minimum score was 06 and the maximum score was 30, the mean score for the test was 15.89 ±0.253, and the mean percentage of knowledge was 52.96%.

Table 3: Comparison of levels of knowledge score in professional education.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
</tr>
<tr>
<td>ANM</td>
<td>98</td>
<td>17.35</td>
<td>3.59</td>
<td>0.36</td>
<td>16.63</td>
<td>18.07</td>
<td>10.00</td>
</tr>
<tr>
<td>GNM</td>
<td>100</td>
<td>13.00</td>
<td>3.61</td>
<td>0.36</td>
<td>12.28</td>
<td>13.72</td>
<td>5.00</td>
</tr>
<tr>
<td>B.Sc. Nursing</td>
<td>98</td>
<td>17.37</td>
<td>4.31</td>
<td>0.43</td>
<td>16.50</td>
<td>18.23</td>
<td>7.00</td>
</tr>
</tbody>
</table>
**Table 3:** Shows that the Mean knowledge score for ANM was 17.35± 3.59, for GNM it was 13.00± 3.61 and for B.Sc. nursing it was 17.37±4.31. The above findings show that B.Sc. Nursing students had good knowledge than ANM and GNM.

**Table 4:** Association of the knowledge score with demographic variables

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1257.115</td>
<td>2</td>
<td>628.558</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>4352.980</td>
<td>293</td>
<td>14.857</td>
<td>42.308</td>
<td>0.000, S</td>
</tr>
<tr>
<td>Total</td>
<td>5610.095</td>
<td>295</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 shows that by using one way ANOVA statistically significant variation was found in knowledge score amongst professional education of three new entrants of the nursing institute (F=42.308, p-value=0.000).

**Table 5:** Multiple Comparisons: Tukey Test

<table>
<thead>
<tr>
<th>Profession</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANM</td>
<td>GNM</td>
<td>4.34</td>
<td>0.548</td>
<td>0.000</td>
<td>3.06</td>
<td>5.64</td>
</tr>
<tr>
<td></td>
<td>B.Sc. Nursing</td>
<td>0.02</td>
<td>0.551</td>
<td>0.999</td>
<td>1.32</td>
<td>1.28</td>
</tr>
<tr>
<td>GNM</td>
<td>ANM</td>
<td>4.34</td>
<td>0.548</td>
<td>0.000</td>
<td>5.64</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>B.Sc. Nursing</td>
<td>4.36</td>
<td>0.548</td>
<td>0.000</td>
<td>5.66</td>
<td>3.08</td>
</tr>
<tr>
<td>B.Sc. Nursing</td>
<td>ANM</td>
<td>0.02</td>
<td>0.551</td>
<td>0.999</td>
<td>1.28</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>GNM</td>
<td>4.36</td>
<td>0.548</td>
<td>0.000</td>
<td>3.08</td>
<td>5.66</td>
</tr>
</tbody>
</table>

Table 5 shows comparing mean knowledge score in all the three new entrants of nursing institute statistically significant difference was found among professional education of three new entrance of nursing institute. Except ANM (P=0.999) shows statistically no significant difference.
Discussion

A descriptive cross-sectional study has been conducted to evaluate newcomers to the health university in India’s knowledge of HIV / AIDS. The goal of the study was to evaluate knowledge of HIV / AIDS among students of newly established medical care and sensitivity among informers as well. The mean knowledge score for the professional education of ANM was 17.35± 3.59, for GNM it was 13.00± 3.61 and for B.Sc. Nursing it was 17.37±4.31. This score shows new entrants B.Sc students were well-known than students of ANM and GNM. By using one way ANOVA statistically significable variation was found in the knowledge score of three new entrances to the nursing institute in professional training as (F=42.308, p-value=0.000). Statistically significant differences between the professional training of three new entrants were found when the mean knowledge score was compared by using the Tukey test.

The National AIDS Control Organization’s (NACO) National Behavioral Surveillance Survey (BSSA) has also reported having higher rates of familiarizations of the terms AIDS (86 percent) than HIV (72 percent) for 78,916 Indian youth (15-24 years). The majority of young people who knew about HIV / AIDS were also aware of the possibilities of transmission of HIV / AIDS through non-protected sexual contacts (92%), infected blood transfusion (95%), and use / infected needles (94%). Over two-fifths of the young people could identify correctly three common misconceptions on HIV / AIDS transmission 6

Sadeghi M, Hakimi H (2008) studied the awareness and attitudes of Iranian dental students towards HIV / AIDS. This cross-sectional survey aimed to evaluate the knowledge and attitudes of Iranian dentists to patients with HIV / AIDS. All 750 dental students who participated in the 10th Dental Student Congress in Isfahan, Iran, were given a 53-point self-administration questionnaire. The total questionnaire response rate was 60.7%. While the majority of students had excellent knowledge (78.4%), only 1% held professional attitudes to treat HIV / AIDS patients 7

Oliveira E.R. et al carried out a related analysis of Brazilian dental students’ awareness and attitudes towards HIV infections. The dental students from the Federal University of Bahia (Brazil) treated patients in university clinics included 250 dental students. The tool consisted of 32 questions pre-coded and two open-ended questions. The findings showed that students’ awareness of HIV / AIDS generally increased as the program progressed, but that they were not compatible with the entire barrier technologies used to control infection and the clinical protocol. Therefore, the perceptions and attitudes of students towards the disease need to be discussed more explicitly. 8

Aggarwal A (2013) carried out an HIV / AIDS-related study between dentists. The purpose of this research, therefore, consisted of assessing the awareness and the actions of HIV / AIDS-related dentists among the 460 students of the Bareilly, Brazilian Institute (UP) of India. The students were conducted with a self-administered survey of 53 standardized questions. The results show that the awareness of students about HIV / AIDS has generally been improved with curricula progression; however, all barrier strategies for infection and clinical protocols have lost continuity and conformity. 9

Conclusion: Students on various professional courses pass a competitive examination to be admitted to the medical professional university. However, with students’ immediate educational background being similar (10+2), three professional streams in this study showed a clear difference in their level of knowledge. Ignorance and mistaken convictions can influence a person’s behavior and communication. However, there is a sufficient chance for thorough awareness, a positive attitude and behavioral improvement to be improved by students during their training time also required greater attention to GNM and ANM students. Although this research is dedicated to evaluating knowledge depth, it has not dealt with other important factors relating to attitude, actions, and practices.

Financial support and sponsorship: Nil.

Conflicts of Interest: None

Acknowledgment: The authors are thankful to all the students who took part in the analysis. Authors are grateful to editorial board members and a team of reviewers of medico legal updates who have helped to bring quality to this manuscript.
References


Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1

Impact of COVID-19 on Women, Pregnancy and Psychologically

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Abstract

Background: Gender differences among the responses to the COVID-19 disease needs to be studied. As pandemic is growing and more and more cases are being registered, correlation between disease and gender response if any should be examined.

Summary: The pandemic of COVID-19 is evolving which reported its first case in Wuhan, PRC, considerable amount and efforts are only directed towards containment of the virus. The effect of the pandemic on pregnant women physiologically, psychologically and classification by socioeconomic status can give better trends to study. Further mortality rate between men and women and reason behind it can be evidently proved after through study. After all, one solution to different problems cannot be implemented. Middle East respiratory syndrome (MERS) and Severe Acute respiratory syndrome (SARS) also belonging to similar family of the coronavirus have more things to study regarding which strategy should be employed in containing the viral spread. Vertical transmission is not seen yet but occurrences of sporadic deaths of infants or still birth could be explained after research.

Conclusion: Gender based case fatality rate and its reasons can be extremely helpful in treating the patient with specific gender. Psychological state in the time of distress always been a cause of worry. It worries more when identified in pregnant state. All these aspects need to be studied thoroughly.

Keywords: COVID-19, OBSTRETICS, INFODEMIC, SARS, MERS, VERICAL TRANSMISSION

Introduction

The COVID-19 disease which first reported in Hubei province of People’s Republic of China in December 2019. Researchers then started studying the continuously evolving virus and found out that its shape resembles to the crown. Hence, they named it coronavirus. The disease caused by the coronavirus strain is known as coronavirus disease 2019 also known as COVID-19. Nearly 33 million cases have been reported and ever increasing. No downward trend has been recorded so far. Minor fluctuations can be seen. Nearly a, million people died of the disease. Symptoms include minor cough, cold and fever to asphyxiation and diarrhea. Various strategies are being implemented and rectified on daily basis. Previous outbreaks of the coronavirus family include severe acute respiratory syndrome (SARS) and middle east respiratory syndrome. World Health Organization (WHO) after seeing the severity and nature of quick spreading decided that it should be declared as pandemic in mid-march 2020. Since then Health authorities and governments are trying out various plans to contain the viral spread. Extreme non-pharmacological interventions like lockdown, movement restrictions, physical distancing, wearing mask were tried out. As time passed there is gradual lifting the ban on the movement restrictions. As consequence the cases of infected patients are increasing rapidly. The rising cases overwhelmed the health infrastructure and left them exposed. As the result of lockdown various persons were confined to homes and non-immediate surgeries were postponed. This includes elderly and pregnant women. Pregnant women need regular checkups between the trimesters. But as almost all the resources were diverted to the containment of COVID-19 other medical departments were left unattended. Due to movement...
restrictions and fear of contracting the disease, pregnant women and their families were refraining the hospital visit. This can create complications as regular checkups are necessary in pregnancy. In chalking out the plan to deal with this previous outbreaks of SARS and MERS can be studies. Vital data needs to be studied so that it can be used in drawing out the plan. Severe restrictions imposed should be studied and define their viability and employing local health care workers should be the part of the plan. Transmission vertically, from mother to offspring is also a crucial topic to be examined. Although the chances of contracting the disease of men and women are same, mortality rate is more in men than women. Women research needed more funding to do more fact-finding study. Habits and innate response to the virus is different in men and women. The neglected and one of the most important topics of psychological state of the pregnant women also be studied. Often it is neglected and leaves a scar on the mind of the patient. Neonatal and postpartum care is necessary in these times of pandemic. In this paper we will study all these aspects in light of the COVID-19. 

Pregnancy and Covid-19

Wuhan where first case reported of the COVID-19 is creating a global disorder in health care sector. Fever, cough and in some cases, diarrhea is reported. Oral route through sneeze and cough are the transmission factors of the disease. After testing the sludge from the residential areas, it is found out that feces of the human also contain the viral strain. As the case with comorbidities, pregnancy is also a vulnerable state with respective COVID-19 infection. While in pregnant state, women undergo immense transformation, mentally as well as physically. The already immunosuppressive state of the person is complemented by other physiological changes like change in hormone level, change in heart rate, respiration and breathing etc., which can be very dangerous if the person is contracted by COVID-19. Already maternal mortality rate (MMR) is highly debatable topic as scientific and health care community is striving hard to bring down the MMR rate. Now this deadly pandemic is only adding to the concerns of the associated professionals. Though there is considerable decline in the MMR, still theumber is far behind the set goal by various policies. In countries like India where there is huge population and population density it is decline to 113 per Hundred thousand live births. The sustainable development goals set by United Nations aims to reduce global mortality rate to 70 per hundred thousand births. At the starting phase of the pandemic, the obstetric patients were categorized as low risk. Then it was rectified as the data becoming available and more research was being done. It was seen from various studies that there was more need to shift the patient in intensive care unit (ICU) than non-obstetric women. However, the death rate remains low on paper until now. This can be attributed to underreporting than good healthcare facilities. The non-pharmacological interventions (NPI) like social distancing, prohibition on roaming and gatherings, persistent lockdowns made situation much worse as pregnant women’s and their families was finding it hard to access the health care facility. There is stark difference can be observed in between socio-economic groups. Poorer people were already experiencing job loss and the added cost of transportation only added to their woes. On authorities’ side, Major chunk of or rather say almost all the resources was being used to mitigate the spread of COVID-19. This creates artificial shortage of health care services for non-COVID-19 patients which also includes elderly who were having surgeries even which were postponed. The regular checkups between trimesters was completely halted. Further there is also a fear of large scale unwarranted births as people were finding it difficult to access the contraception’s and necessary medicines.

COMPARING WITH SARS AND MERS

The coronavirus disease 2019 also known as COVID-19 is caused due to the coronavirus family. The shape of the virus resembles with the crow so named as coronavirus. It belongs to the family whose other members caused the severe acute respiratory syndrome (SARS) and Middle Eastern respiratory syndrome (MERS) earlier in 2002 and 2012 respectively. Taking cue from that we can somehow predict the relation between pregnancy in females and the effects of COVID-19 on it. The new form of coronavirus popularly known as novel coronavirus (nCOV). The similarities observed are incubation period. All three of them have incubation period ranging from two days to fourteen days. Also, bat is the common animal hosts and almost all reported cases were from adults excepting very rare occurrence of child case report. Almost 95 percent patients’ symptoms
were fever and cough in all the three-virus infection. In addition to these symptoms some rare cases of diarrhea also have reported. Symptoms part can be considered dynamic because in some form or the other they had changed. \(^{(4)}\)

SARS which reported 8000 cases worldwide has similar symptoms. Also like in COVID-19 superspreading also caused widespread infection. Highest possibility of viral excretion was in the second week of the infection. Hong Kong was the place where largest number of pregnant infected cases were found. Out of 12 cases of SARS infection in pregnant women 3 died, making case fatality rate to 25 percent. Respiratory distress syndrome was prevalent among them. Abortion, premature or preterm delivery of infant was seen as the consequence of SARS infection. Half of the in first trimester and others were in second trimester. \(^{(7)}\) Two of the new born babies were suffering from respiratory distress syndrome. Two babies were having gastrointestinal infection. But the correlation between mother’s infection and infant’s disease was not established by the empirical evidence. Another interesting yet predictable conclusion was that forty percent of pregnant women was put on ventilator while this figure drops to thirteen in case of non-pregnant. Case fatality rate was also high among pregnant women’s than non-pregnant ones. Also, other non-pharmacological measures were at place to supplement the efforts. Some lessons can be learnt to tackle the COVID-19.\(^{(8)}\)

MERS also known as Middle East respiratory syndrome was first identified in the Persian Gulf countries. In 2012 when the outbreak reported there were almost two and half thousand cases reported of which around 900 deaths were reported. The case fatality rate stands at around 38 percent. Same initial symptoms of cough and fever were observed. Co-morbidity is also a factor in deciding the severity of the cases.\(^{(9)}\) Not much study has been done but there were thirteen cases of pregnant women who have been infected with MERS. Out of which 3 died. The case fatality rate in pregnant women stands at 23 percent. Contact tracing helps finding two women in asymptomatic state. Complications in the delivery is observed but no correlation is established with the infection.\(^{(2,10)}\)

**Non-pharmacological interventions and pregnant women.**

As the outbreak turned pandemic is wreaking havoc in the entire world little was known about the containment of the disease. After clarifying the WHO stated that the human to human transmission is highly relevant in this disease. Naturally various agencies and governments started to act. They put restrictions on movements of the people all of a sudden as this pandemic has not given enough time to strategize. So, government plans motive was to break the chain and identify the people who are already infected. Non-pharmacological interventions (NPI) such as physical distancing, wearing mask which is also called as social vaccine, lockdowns, movement restrictions etc. was imposed. This severely halted all other things except the COVID-19 containment. All the resources were targeted to serve the COVID-19 containment purpose. \(^{(11)}\) Naturally people avoided the health services due to fear. This also include the pregnant women which needs regular checkups and vaccination. This severely impacted the necessary interventions by the health care professionals which can lead to other complications other than the COVID-19 infection. The immediate family of the pregnant women were also in fear of attracting the disease of services are accessed. Ground and local health care workers need to look into the matter as soon as possible so the other disaster couldn’t unfold. A very sad impacts on women in general is that they are very prone to domestic violence due to forced close consistent proximity with their partner. Also, as part of NPI all shops including those who serves liquor etc. are also closed down. Addicted people release their anxiety either on their children’s or their spouse. According to the UN study there are chances of seven million unwanted pregnancies due to lack of access to contraceptives. Various programmed run by non-governmental organization has halted their programmers which was crucial in creating awareness in rural masses.\(^{(12,13)}\) Infodemic is also a serious issue to tackle. Only considering pregnant ladies, false information which turns into infodemic is creating fear about hospital visits and other fears of falsely showing positive with infection. \(^{(3)}\) This hinders the need of regular visit to the doctor.
TRANSMISSION FROM MOTHER TO OFFSPRING

There is not much empirical evidence whether the mother to offspring transmission of the disease takes place or not internally. Few positive cases of the mothers who delivered babies were found out that there was no prenatal transmission of the disease. But some instances of few days old babies testing positive for the novel coronavirus disease is concerning. But it can be attributed to the transmission outside the womb where symptomatic mother accidently sneezes or coughs and droplets inhaled by the baby. No infant death has been reported as far now.\(^\text{(9)}\) Transmission through breastfeeding is also a contentious topic but no evidence on this also found. But coughing or sneezing near the baby can be very dangerous and can cause infection. Proper masks to be wear at the time when baby is in near proximity. \(^\text{(8)}\)

LOW MORTALITY RATE AMONG WOMEN

Figures till date shows that men have more case fatality rate (CFR) than women in COVID-19. This means more men are dying than women after contracting the COVID-19. This is interesting to study as it indicates towards manifestation of disease in different way. Immune response can also be a reason among others. Also, women are less prone to engaging in risky behavior, less sin goods consumption and more sincere in following the non-pharmacological interventions such as wearing masks and physical distancing.\(^\text{(14)}\) More funding overall and more research needed in the women health research which is already underfunded. Correlation between how a disease behaves in men’s anatomy and women’s anatomy should be established. Then only we can conclude that one size fits all approach is not efficient in dealing with all the genders. Although men and women are equally prone to contracting the disease. \(^\text{(15)}\) Comorbidities are the main factor in confirming the severity of the disease. It is found in study that men have more heart ailment than women which is one of the prime reasons in deciding the case fatality rate. Diseases related to blood and liver caused by excessive consumption of alcohol also found in men mostly. Smoking is an activity which very few women subscribe to leading to their lungs being more efficient than men who are more in to that activity. Larger percentage of angiotensin-converting enzyme 2 (ACE2) in the blood of the men than women is also a cause of concern. Since ACE2 let the coronavirus infect the healthy cells it can be attributed to why more men are vulnerable than women. \(^\text{(2)}\)

PSYCHOLOGICAL IMPACT OF THE COVID-19

In the time of lockdown and movement restrictions due to fast spread of the COVID-19, there was distress everywhere. Slowly the anxiety built up and much needed events to vent out the anxiety like social gatherings and festivals were not happening. It creates a mental condition where you feel low and tired due to the monotonous life that just has started. The rising uncertainty accumulate overtime. It further worsens the situation because person do not know when this situation will end. And constant fear of catching the disease is there already. Home confinement also leads to less physical activity and more lethargy which then transforms into mental tiredness. If the family member is working on frontline to fight the disease then there is additional safety concern about the lover ones. It affects all age group and physiological condition. But among most vulnerable pregnant women are at the forefront. First, they are already bedridden and in later days they are unable to go out on their own. Second the loved ones visiting them are no more near them to bear the pain of the pregnancy. The hormonal imbalance which is common in the pregnancy aggravates the situation more.\(^\text{(5)}\) This leads to unnecessary thoughts of fetal deaths and vertical transmission worries the person more. Psychological interventions are as important as clinical intervention. Especially when there is the question of mental wellbeing of the pregnant women. It deeply affects the state of the neonates and affects their full development. Study showed that psychological impact of the COVID-19 is more in first trimester pregnant women than in second and third trimester pregnant women. Almost 70 percent women in pregnant state with infection showed more than normal anxiety. Around 50 percent women showed anxiety about vertical transmission of the disease from them to the offspring. Socioeconomic status of the pregnant women also matters in her pregnancy. Women from lower strata of the socioeconomic ladder has to work even if they are ill. Pregnancy in poor rural household already lacking the regular public health center forget
about the secondary and tertiary health service which almost all are concentrated in urban areas. NPI halted this access to. Domestic violence is on rise as lockdowns and movement restrictions are imposed. Addictive urge is not fulfilled so people are venting out their anger to the family members. Urban slums have bare minimum facilities with common sanitation and water facility. This create a huge challenge in the containment as there is congestion and therefore unable to implement the physical distancing measures (16,17,18). So far now, social distancing and isolation of suspected cases are the best prevention strategies for Covid-19(19,20).

**Conclusion**

With almost 33 million and increasing cases of the COVID-19 infection reported, governments are struggling to at least contain the virus spread. The highly uncertain behavior with changing viral strain and symptoms among the patients making it difficult for the scientists, researchers and health care professionals to chalk out the containment strategy. Among various of patients, pregnant women are one of the most vulnerable groups. Although some deaths are reported in pregnant women infected with COVID-19 and reports of still births, abortion, there is need conclusive study which clearly established the correlation between the COVID-19 infection and effects of it on pregnant women. Previous outbreaks of SARS and MERS can guide us on the plan to avoid any complications in pregnant women with the afore mention infection. The effects of non-pharmacological interventions like physical distancing and lockdown had severe impact in halting the access to healthcare service to the pregnant women. There is a need of local healthcare workers and professionals to reach out to the future mothers who needs medical intervention for vaccination and regular checkups. Also, no access to contraception’s will lead to many unwanted pregnancies which can be a challenge in upcoming days. Socioeconomic status plays a crucial role in any disaster as they are less resilient in terms of health and economic condition. Especially women where they are the victims of domestic violence and poor health and sanitation facilities. A common and widely seen factor was severe anxiety about the vertical transmission of the disease which is attributed to the innate response of the human beings toward the safety of their offspring. Proper guidance to the treating doctors or other health care professionals to counsel and give support to the future mothers. Designated and experienced doctors related to the psychological departments can be employed in the maternity ward. More comprehensive study needed with the empirical evidences to find and establish the correlation between pregnancy and COVID-19. Telemedicine option should be implemented wherever necessary. Also, pregnant women need not withhold the vital information of fever, cough due to fear of contracting the virus. Hygiene and sanitation should be assessed and rectified which helps in preventing the virus spread. Monitoring the mother and offspring in post-partum period is essential. Study by gender specific fatalities needed to establish or to undisclosed the reason behind high mortality rate among men than women. Gender specific habits needs to be monitored.

**Ethical Approval:** IEC, DMIMS Wardha

**Conflict of Interest:** None

**Funding:** DMIMS, Wardha

**Reference**


Assess the Prevalence of Hypercalcemia among Immobilized Patients

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Abstract

Immobilization hypercalcemia is a diagnosis in which excessive bone metabolism abnormalities and conditions related to reduced movement such as medullar lesions or vascular cases. Investigation needs a more evaluation to find out other causes of hypercalcemia. Incidence rate of immobilization hypercalcemia is 11-22% in spinal cord injury group and 20-30% in immobilized patients secondary to fractures in 2 case series. Usually it occurs 4-6 weeks post trauma (1 week-16 weeks) may stay elevated for up to 12 months based on immobilization. Aim-To determine the prevalence of hypercalcemia among immobilized patients.

Materials and Methods-An analytical research approach and a cross-sectional research design were used. The study was conducted in Acharya Vinoba Bhave Rural Hospital; Sawangi Meghe Wardha. The samples were selected using purposive sampling technique, sample size was 30 and blood test done for screening after written informed consent. There were total 30 patients, blood samples which were carried out during study period, it was concluded that out of 30 immobilized patients 14 patients is having hypercalcemia in immobilized state. The study found that there was a person suffering from hypercalcemia in their immobilized state. Hence this study helps in treatment of hypercalcemia during their immobilized state of the patients. From this study it is concluded that 46.67% of the patients suffering from hypercalcemia.

Key Words: disabled patients, high level of calcium in the blood, pervasiveness.

Introduction

Hypercalcemia in immobilization was first discovered by Albright et al. in 1941 and thought that it may occurs due to excessive bone resorption. It is an under-recognized because of increased calcium level in the blood specially in hospitalized bedridden patients.¹ Immobilization hypercalcemia is a diagnosis in which excessive bone metabolism defects and conditions correlated with reduced mobility like lesions of the medulla or vascular injury. Investigation needs further study to find out certain sources of hypercalcemia.²

Incidence rate of immobilization hypercalcemia is 11-22% in spinal cord injury group and 20-30% in immobilized patients secondary to fractures in 2 case series. Usually it occurs 4-6 weeks post trauma (1 week-16 weeks) may stay elevated for up to 12 months depending upon mobilization.³

Diagnosis of immobilization hypercalcemia was verified by elevated urinary excretion of pyridinium cross-link’s-urine pyridinoline / creatinine ratio 386 nmol / mmol (NR 5-21.8). The hypercalcemia returned to health within one week with intravenous fluids and intravenous pamidronate (90 mg) and Normocalcaemia was sustained throughout the next several weeks until mobilization started. When analyzing the history and ignoring any factors, the elevated degree of hypercalcemia will seem to be linked with extreme trauma and sustained immobility.⁴

Hypercalcemia with immobilization induces a rise in the resorption of osteoclast bones. The cascade of events associating the lack of mechanical stress on the bone with increased resorption can include altered piezoelectric effects in the bone. The subsequent immobilization attributable to trauma, severe damage to the spinal cord or some other cause in this situation promotes the resorption of osteoclastic bone. Because of this condition increased
calcium in the urine and calcium loss from the bones may occur. Increased calcium level in the blood occurs whether calcium efflux becomes increase or the glomerular renal filtration volume becomes decreased. Muscle movement spreads a bone development signal through the osteocyte. With prolonged inactivity of the patients, the mechanical stimulation for bone development triggered by less muscle movement, leaving resorption unopposed. After immobilization the bone resorption occurs within 18 months, even after patients begin remobilization. Ultimately the resorption contributes to osteoporosis; particularly of the appendicular skeleton. The calcium produced by bone remodeling is excreted through the renal tubules. After injury hypercalciuria takes place within the first week and continues for 6-18 months. Calcium release cuts off parathyroid hormone development after few weeks of spinal cord injury. Decreased Parathroid hormone is related with excessive serum phosphate concentrations and decreased secretion of 1, 25-dihydroxyvitamin D. If the rate of calcium resorption increased the capacity of urinary excretion, hypercalcemia occurs. This problem is mostly common in children and young adolescence and patients with renal diseases. Hypercalcemia typically begins 4-8 weeks after immobilization but may start as early as 2 weeks or as late as 6 months after the injury. 

Immobilized hypercalcemia develop due loss of mechanical stress, it leads to excessive resorption of osteoclast bone and reduced the formation of osteoblast bone and then excessive calcium for normal regulatory mechanism of the body.

In symptomatic patients serum calcium level is more than 11.5-12 mg/dl. Clinical manifestation of hypercalcemia include tiredness, weakness, lethargy, pain in abdomen, difficulty to passing faeces, loss of appetite, vomiting, excessive thirst, excessive urination, and dryness. Patients may also show behavioral problems, tiredness, lethargy, misunderstanding or an acute psychosis. Early care has to be based on early mobilization, hydration with intravenous (IV) normal saline, rebuilding of the stability among calcium elimination and resorption and loop diuretics; zoledronic acid has to start if there is no progressive condition. If treatment not given, patients can develop dehydration, behavioral disorder, calcium oxalate nephrolithiasis, and chronic kidney diseases.

**Aim:** To determine the prevalence of hypercalcemia among immobilized patients.

**Materials and Methods**

An analytical research approach and a cross-sectional research design were used. The study was conducted during December 2019 and the setting was selected in Acharya Vinoba Bhave rural hospital Sawangi (M) Wardha city after getting ethical permission (Ref. no: DMIMS (DU)/IEC/Aug-2019/8206 ON DATED 03/10/2019, By using purposive sampling technique, 30 immobilized patients were selected from the Acharya Vinoba Bhave Rural Hospital Sawangi (M) Wardha based on the calculation.

**Statistical Analysis**

The demographic data, collected and descriptive analysis was done in terms of frequency and percentage. For statistical analysis SPSS version 16.0 was used.

**Method of data collection:**

**Section A:** Consist of demographic characteristics of sample such as age, gender, diagnosis, and calcium level.

**Section B:** blood test done for checking calcium level.

**Methodology**

Target population (Immobilized patients)

Accessible population (Immobilized patients in AVBRH Sawangi (M) wardha)

Purposive sampling technique used and sample size was 30

**Data collection**

**Result**

Table no. 1 showed that 3.33% of the patients were in the age range of 20-29 years, 13.33% of them were in the age range of 30-39 years, each 30% of the patients were in the age range of 40-49 years and 60-69 years, 13.33% were in the age range of 50-59 years and 10% of them were belonging to the age group of more than 70 years.
Table no.2 showed that 73.33% of the patients were males and 26.67% of them were females.

Table no.3 showed that each 6.67% of them patients had cellulites, ulcer over foot, and fracture of patella, 10% of patients had sub arachnoidshemorrhage and each 3.33% of the patients had brain tumor, traumatic cervical spine respectively.

Table no.4 showed that 53.33% of the immobilized patients were having normal calcium level and 46.67% of the immobilized patients were having abnormal calcium level.

**Table 1: Distribution of patients according to their age**

(n=30)

<table>
<thead>
<tr>
<th>Age Group(yrs)</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29 yrs</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>30-39 yrs</td>
<td>4</td>
<td>13.33</td>
</tr>
<tr>
<td>40-49 yrs</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>50-59 yrs</td>
<td>4</td>
<td>13.33</td>
</tr>
<tr>
<td>60-69 yrs</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>≥70 yrs</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean±SD 51.93 ± 15.26

Range 20-87 years

**Table 2: Distribution of patients according to their gender**

(n=30)

<table>
<thead>
<tr>
<th>Gender</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22</td>
<td>73.33</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>26.67</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3: Distribution of patients according to diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulitis</td>
<td>2</td>
<td>6.67</td>
</tr>
<tr>
<td>Brain Tumour</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Cerebro vascular episode with SDH</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>CKD</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Colonic ulcer with diselectrolytimia</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Compound grade fracture left tibia and fibula</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Compression fracture l1</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>D12 fracture with neonadeficit</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Fracture of patella</td>
<td>2</td>
<td>6.67</td>
</tr>
<tr>
<td>Head trauma</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Hemmorage contusion</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Hepatomegaly with ascites</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Hypertention With CerebelarHemmorage</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Intertrochanic Fracture Right Femur</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Neurologous Disorder With Bedsore</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Pathological Fracture</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Pontine Abscess</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Prolapsed Intervental Disc</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Proximal 3rd Tibia Right Side</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>PsoriatriArthritis Affecting Both Hip</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Spondyiosisthesis</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Sub Arachnoid Hemorrahge</td>
<td>3</td>
<td>10.00</td>
</tr>
<tr>
<td>Traumatic Cervical Spine</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Ulcer over foot</td>
<td>2</td>
<td>6.67</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>
### Table 4: Distribution of patients according to calcium level (n=30)

<table>
<thead>
<tr>
<th>Calcium level</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8.5-10.5 mg/dl)</td>
<td>16</td>
<td>53.33</td>
</tr>
<tr>
<td>Abnormal (&gt;10.5)</td>
<td>14</td>
<td>46.67</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>8.30±0.70(6.60-9.40 mg/dl)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The following study supported the present study by Kyung Ae Lee and Wan He Yoo concluded one case report on Immobilization Hypercalcemia-Associated Acute Renal Failure in a Patient with Chronic Tophaceous Gout. They reported that hypercalcemia with immobilization is a uncommon source of severe kidney dysfunction in patients with immobilized state related to restricted motion. Researcher suggested that immobilization may be a probable source of hypercalcemia-prompted serious kidney problems in patients with chronic Tophaceous gout involving several large joints, and professional preparedness is necessary to avoid excessive evaluations and lethal problems.

Another research study concluded that excessive resorption of osteoclastic bone is the key incident for developing the immobilization hypercalcemia. The suggested pathophysiological cause is a loss of mechanical tension due to the absence of loading and muscle activity, low vascularity, metabolic changes in bone and bone denervation.

One case report showed that a 19-year-old elderly patient detected with hypercalcemia after investigation by a family doctor. The patient was suffering from quadriplegia because of spinal cord injury after falling into surface water. Patient had remained immobilized for 3 years. At the time presentation, the patient developed specific signs related to hypercalcemia like lethargy, sickness, quiescence, and loss of appetite, difficulty to passing the stool and thirst. After inspection, bed sore was present in the sacral bone. Reduced hemoglobin level was reported in laboratory workup. In comparison, calcium level was increased although intact parathyroid hormone and 25-OH vitamin D was lower than average. Calcium in urine was also elevated in 24 hours.

Another research study showed that a 48-year-old elderly patient experienced a pronounced and chronic hypercalcemia 3 months after admission for paraplegia arising from extreme peripheral neuropathy most possibly of alcoholic etiology. Serum ionized calcium was increased, and parathyroid hormone levels were low normal by the two separate radioimmunoassay. Urinary calcium excretion was markedly increased and serum 1, 25-dihydroxy vitamin D level was decreased. A comprehensive clinical review of potential occult
malignancy, myeloma, and sarcoidosis as a source of hypercalcemia did not show any promising outcomes. Calcitonin therapy started for the plasma calcium to be rapidly elevated, and its withdrawal culminated in reappearance of hypercalcemia. The patient started intensive physical rehabilitation for relief of the neuropathy and then the physician gradually decreased Calcitonin therapy, and the serum calcium of the patient stayed normal for the next 11 months.\(^9\)

Excessive resorption of the bone marks in hypercalciuria in days and hypercalcemia a few weeks later. If the ability of the renal to eliminate calcium is overdid. Hence kidney inadequacy raises the threat of prolonged inactivity due to hypercalcemia. Children and young adults are more common because of their increase bone turnover.\(^4\)

One study showed that a 79-year elderly patient with chronic kidney disease stage four was admitted with a limited-weight-bearing status after right-hip arthroplasty. Patient developed hypercalcemia (11.5 mg/dL) with serum albumin of 2.5 g/dL after four weeks. Despite IV fluids, hypercalcemia worsened (corrected serum calcium, 14.5 mg/dL), and she was rehospitalized.\(^10\)

Recommendation

1. Similar study can be conducted with more study participant to generalize the findings.

2. A study may be conducted on staff nurses to assess the knowledge regarding complication of immobilization.

Conclusion

Immobilization hypercalcemia more commonly impacts the teen age groups associated to the rapid bone turnover that accompanies development. Males are more commonly affected than females because of their more bone build. Immobilization hypercalcemia is common in tetraplegia patients than paraplegia. Immobilization hypercalcemia is a diagnosis in which excessive bone metabolism defects and conditions correlated with reduced mobility like lesions of the medulla or vascular injury. Investigation needs further study to find out certain sources of hypercalcemia. Present study findings will helpful for preventing the further complication and based on the above cited findings, it is concluded that it is effective method to determine the calcium levels in patients and reduces the risk for hypercalcemia among immobilized patients.

Acknowledgements: We would like to thank the authors whose works have cited and included in this study such as Gopal H, Sklar AH, Sherrard DJ, Gallacher SJ, Ralston SH, Dryburgh FJ, Kaul S, and Sockalosky JJ . We acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. We are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Source of Funding: No funding

Conflict of Interest - Nil

References


10. Booth KA, Hays CI. Using Denosumab to Treat Immobilization Hypercalcemia in a Post-Acute Care Patient. J Clin Endocrinol Metab. 2014 Oct 1;99(10):3531–5. A number of studies in this region were reviewed which have direct or indirect effects on acquisition or progression of hypercalcemia.


High Risk Population For COVID 19

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Abstract

COVID-19 is a new type of corona virus that causes the disease. ‘CO’ is for corona, ‘VI’ for viruses, and ‘D’ for illness. This disease was previously referred to as ‘2019 novel corona virus’ or ‘2019-nCoV’. Corona virus is the contagious disease (COVID-19) is arising from newly discovered. The COVID-19 virus gets people of all ages infected. However, research to date shows that two classes of people are at greater risk of being strongly struck by COVID-19. The older people and others with existing health problems. WHO stresses that, in order to protect others, everyone must protect themselves from COVID-19. Old age people and individuals with underlying disabilities such as heart problems, asthma, chronic lung disease and cancer are more vulnerable to severe disease. Men is at a higher risk than females for COVID-19. **Conclusion** According to this short communication article, two groups of people are at higher risk of developing severe COVID-19 disease. Old age people and individuals with underlying disabilities such as heart problems, asthma, chronic lung disease and cancer are more vulnerable to severe disease. These peoples have to take more precaution to prevent covid-19 infections.

**Key words**- chronic respiratory disease, elderly people, greater risk people, heart disease, 2019 –novel corona virus

Introduction

COVID-19 is a new type of corona virus that causes the disease. ‘CO’ is for corona, ‘VI’ for viruses, and ‘D’ for illness. This disease was previously referred to as ‘2019 novel corona virus’ or ‘2019-nCoV’.¹ In more than 200 countries in the world, the Corona Virus Outbreak 2019 (COVID-19) pandemic is the latest epidemic.³⁶ The COVID-19 is a rapid succession pandemic. Fear has taken control of the whole planet. The number of those afflicted has risen and the death toll has reached a disturbing level. In this time of crisis, competent management of the afflicted, regular deaths, self-care and quarantines requires a lot of bravery to face the reality.³⁷

Corona virus is the contagious disease (COVID-19) is arising from newly discovered. The COVID-19 virus gets people of all ages infected. However, research to date shows that two classes of people are at greater risk of being strongly struck by COVID-19. The older people and others with existing health problems. WHO stresses that, in order to protect others, everyone must protect themselves from COVID-19. Old age people and individuals with underlying disabilities such as heart problems, asthma, chronic lung disease and cancer are more vulnerable to severe disease. Men is at a higher risk than females for COVID-19.²

Corona virus disease (COVID-19) is a newly discovered corona virus-related contagious disease. Many Individuals contaminated with COVID-19 may succeed mild to moderate respiratory problems and recover without having to take any special care. If a individual infected has cough or sneezes, virus COVID-19 diffused mainly by droplets of saliva or nasal flushing, so it is necessary to practice the respiratory good manners (such as coughing with a bend elbow).³

COVID-19 is a corona virus outbreak that originally occurred in Wuhan, Hubei Province, China in December 2019 but has now grown into a rapidly spreading pandemic across the world.⁴

As of 17 April 2020, more than 2 Mio. COVID-19 cases have now been recorded to WHO , and more than
135,000 people have lost their lives.  

13,835 confirmed cases in India with 452 deaths by the Ministry of Health and Family Welfare, there are 11,616 active cases with 1,766 cured/discharged cases. (Ministry of Health and Family Welfare 2020)

The COVID-19 virus gets people of all ages infected. However, research to date shows that two classes of people are at greater risk of being highly influenced by COVID-19. There are older people and others healthcare problems underlying them. WHO stresses that everyone must shield himself from COVID-19 to shield others.

The illness causes respiratory disease (such as flu) with symptoms like cough, nausea, and trouble breathing in more extreme cases. We should protect ourselves by constantly washing our hands, avoiding touching our face and eviting near touch with the unwell people (1 meter or 3 feet)

Elderly people and those with underlying medical problems such as heart disease, diabetic mellitus, chronic lung disease and cancer are more likely to develop serious diseases.

Italian data corroborate population groups previously identified at higher risk of serious illness and death. Such categories are elderly people over 70 years old and persons having medical conditions such as obesity, diabetic mellitus, chronic lung disease and cancer. Men is at a higher risk in certain classes than females. Chronic obstructive pulmonary disease.

There are different parts of our population that are most susceptible to COVID-19 secondary severe acute respiratory infection (SARI). Which include the following.

Old age people more than 65 years

An older adult, aged 65 and older people are at increased risk for severe COVID-19 disease and the capacity of their immune systems to combat infection declines and thus make us more susceptible. Coronavirus might affect any community the elderly people is the greater risk of serious illness. 8 out of 10 deaths recorded in the United States occurred in adult’s age of 65 years and older death threat is the highest for those ages of 85 years or older. Older adult Immunology are becoming weaker with age, making it more difficult to fend off infections. Older adults more often have chronic deaths which may raise the risk of serious diseases.

More than 95% deaths occurred in 60 years or older people. More than 50% deaths occurred in 80 years or older. One positive report showed that those people more than age of 100 years who were admitted to hospitals for COVID-19 and now they have completely recovered. It’s clearer that the healthier people were before the pandemic plays a crucial role. Those aged people who are healthily are less at risk.

Researcher concluded that with elderly people, the body has fewer T cells, which has the property for producing virus-fighting chemicals. By adolescence the thymus gland is producing tenfold fewer T cells than it did in childhood, by age 40 or 50, there is another tenfold drop.

Research study suggested that more than 60 years elderly people and specially those aged more than 65 years old are at significantly higher risk of severe disease, requiring respiratory support, and death from Covid-19 than younger age groups.

During the disease or when in quarantine, older adults, those who are in isolation who have the neurological disability may have anxious, afraid, worried, irritated, frustrated, angry who withdrawn. Using informal networks such as social services and health care providers to provide psychological assistance. Educate them about COVID-19 in a straightforward way and show them how elderly people with / without cognitive disability can reduce the risk of infection in their own words.

Aged people with heart disease are younger than 62 years of age or more than 80 years of age.

Individuals with underlying medical conditions:

Serious heart problems

Severe heart complications, like heart failure, coronary artery disease, cardiac parenthood disorder, cardiomyopathy and lung hypertension, can present a higher risk to people from COVID-19 for serious illness. Unlike other viral illnesses including flu, COVID-19 can disrupt the respiratory system and make it more difficult
for the heart to function. For people with cardiac insufficiency and other severe specifications.\textsuperscript{9}

Observations may clarify Older men, tobacco consumers / smokers and those with hypertension are vulnerable also illustrate the significance of recognizing smoking as a possible risk category for COVID-19. COVID-19 may develop serious heart problems including cardiac failure, myocardial infarction and embolism it’s may cause cerebro vascular accidents. They also caution that treatments with COVID-19 may interact with drugs used to manage existing heart conditions in patients.\textsuperscript{13}

Researcher point out that, some conflicting theories about antihypertensive medication making patients susceptible to the Novel Corona virus. Be assured that common antihypertensive medication not raise patients’ risk of infection. It is advised not to stop the antihypertensive medication, hence hypertension is a higher risk factor and it may lead to more complications in case of infection.”\textsuperscript{14}

Researcher evaluated that 45 recent reports pertaining to COVID-19 and cardiovascular complications and found that the corona virus can cause lasting heart impairments. Many patients who develop severe COVID-19 complications already have underlying heart issues. Researcher evaluated that over 72,000 patients with COVID-19 found that about 22 percent of patients who died had cardiovascular co morbidities.\textsuperscript{15}

Research study showed that those patients who were suffering from cardiovascular problems and/or development of acute myocardial injury are associated with significantly worse outcome in covid 19 patients.\textsuperscript{16}

**Lung diseases** – Chronic respiratory obstructive disease (COPD), asthma, lung fibrosis and cystic fibrosis. People has chronic lung disorders are suffering from inadequate ventilation due to a number of conditions which include inflammation, blocked or airways clogged, alveolar defects and other structural problems. Viral diseases exasperate the lung disease at the root and can cause breathing failure, septicemia, and acute respiratory distress syndrome (ARDS). \textsuperscript{9}

Meta-analysis concluded that those patients who having a history of COPD, they are more prone to get covid -19 infection. Encouraged to patients for adopting more restrictive measures for minimizing potential exposure to SARS-CoV-2 and contact with suspected or confirmed cases of COVID-19. \textsuperscript{17}

American Lung Association concluded that above 35 million Americans suffering from chronic lung disease, and researcher said that these patients having greater risk for severe implications if they were to contact the virus.\textsuperscript{18}

Corona virus disease 2019 (COVID-19) can cause respiratory, physical, and mental dysfunction in patients. Hence, pulmonary rehabilitation is important for both admitted and discharged patients of COVID-19.\textsuperscript{19}

Researcher said that those patients who having chronic respiratory problems these patients has to take extra precaution during the middle of COVID-19. “Because the virus attacks the lungs, patients with chronic lung disease such as asthma, COPD, and pulmonary fibrosis are more susceptible to the COVID-19 infection,”\textsuperscript{18}

**Diabetes**

Diabetes, in that dependent, independent diabetic mellitus or diabetic in pregnancy may put people at increased risk of COVID-19 serious illness. Hyperglycemia, or impaired blood glucose regulation, is affecting the immune system. Vascular failure may impede inflammatory reaction locally and antibiotic supersorption.\textsuperscript{9}

Those patients who are suffering from diabetes are among those greater-risk categories that, when they get the virus, can become seriously ill. \textsuperscript{20}

Infection with COVID-19 is a double challenge for diabetes patients.\textsuperscript{21}

Hospitalization and mortality rate of the COVID-19 infection is increased due to diabetes.\textsuperscript{22} comparative study concluded that ICU and non ICU patients with COVID-19 The incidence of intensive care patients with diabetes appears to be double increasing.\textsuperscript{23} Mortality in people with diabetes seems to be about threefold higher compared to the general COVID-19 mortality in China.\textsuperscript{22,23}
Those patients who having diabetes are indeed a greater-risk group of serious illnesses. In the previous SARS, diabetic mellitus was also a causative factor for serious illness and death.  

Statistics indicate that India has the second largest number of people with Diabetes globally. In the current scenario of lockdown, while we all are taking necessary precautions like social distancing and staying home to prevent the rapid spread of COVID-19, managing Diabetes may seem quite challenging.

People with Diabetes are not more likely to get COVID-19 than the general population. However, COVID-19 can cause more severe symptoms and complications in some people living with Diabetes.

**Immunocompromised**

Some conditions and treatments can lead to a person’s immune system weakened, including treatment for cancer, bone marrow or liver transplants, HIV immune deficiencies with or without small cell count CD4, and excessive usage of corticosteroids and other immunodeficiency’s drugs. People with compromised immune systems have reduced their ability to fight infectious diseases.

**Who is immunocompromised is at greater risk of respiratory infections than the average person.**

Research concluded that elderly people with certain co-morbidities and those with immunocompromised may be at greater risk of succeeding severe illness. An immune system helps the body to fight off infections. Hence it is important that the immune system is working efficiently to fight infection such as that given by the SARS-CoV-2.

One case report concluded that COVID-19 affecting an immunocompromised women. A 60 yrs women was admitted to the emergency department on 10 March 2020 due to breathing difficulty and high grade fever, consistent with COVID-19.

**Chronic renal disease treated with dialysis**

Chronic dialysis-treated renal disease can increase a person’s risk for serious COVID-19 illness. Patients with dialysis are more vulnerable to infection and serious illness due to compromised immune systems; medications and kidney failure control procedures; and conditions which coexist like diabetes. Those patients who were suffering from renal disease and other severe associated conditions are at greater risk for more severe illness.

Dialysis patients and those patients who are on immunosuppressive medicines may have weaker immune systems, making it harder to fight infections.

**High Obesity**

Extreme adiposity identified as 40 Body Mass Index (BMI) or higher, puts people at greater risk of COVID-19 complications.

Extreme obesity raises the likelihood of a severe respiration condition called Acute Respiratory Failure Syndrome (ARDS), which is a significant complication of COVID-19 and may cause problems with the ability of a doctor to provide critically ill patients with respiratory assistance. Individuals with extreme adiposity can have several serious chronic conditions and underlying health problems which may increase. A study of 3,615 COVID-19 patients who visited an academic hospital in New York shows that obesity even among relatively younger patients has increased the risk of severity. For example, patients who were extremely obese (BMI > 34 kg / m2) and were under 60 years of age were 3.6 times more likely to be admitted to ICU than patients in the same age group who had less than 30 years of BMI.

In China, data showed from 383 patients having obesity was associated with a 142% greater risk of succeeding severe pneumonia associated with COVID-19. One study showed that more than 4,000 patients with COVID-19 in New York City found that severe obesity was a higher risk factor for hospitalization, 85% of obesity patient’s required mechanical ventilation, compared with 64% of patients without the disease. In addition, 62% of obesity patients died from COVID-19 compared to 36% of those without obesity. Evidence to date suggests that obesity is associated with a greater risk of succeeding severe corona virus disease symptoms and complications independent of other diseases such as heart disease.
Liver Diseases

People with liver disease may have a higher risk of COVID-19 severe disease. Chronic hepatitis like cirrhosis of liver may cause increase the risk of serious COVID-19 disease.9

A recent publication concluded that 54 percent of patients in a single center in China hospitalized for COVID-19 had elevated gamma-glutamyl transferase (GGT). The expression ACE2 is enriched in cholangiocytes, indicating that COVID-19 may potentially cause a higher risk of biliary injury over hepatocyte injury, as evidenced by these elevations of the GGT.33

Cohort study concluded that 1,099 cases of COVID-19 in China showed that 21 (2.1%) had pre-existing hepatitis B. The overall ALT rise was 21.3 per cent (158/741) and the AST rise 22.2 per cent (168/757) respectively. Extreme patients have a higher likelihood of elevation of ALT relative to non-serious patients (28.1% vs. 19.8%) and elevation of AST (39.4% vs. 18.2%). 10.5 per cent (76/722) of patients in total 50.34

Pregnancy – – There is insufficient information about risk and transmission during pregnancy and breast feeding at the time of this writing.9

One research study found that a sign of placental damage in pregnant women with COVID-19, this damage actually affects birth outcomes. Most women with the novel corona virus who had these abnormalities gave birth to healthy babies’ at-term. Pregnant women those who were more than 35 years old , obesity and those who having pre-existing medical conditions , like hypertension and diabetic mellitus, were also at higher risk of occurring serious illness.35

Conclusion

According to the short communication, two groups of people are at higher risk of developing severe COVID-19 disease. Old age people and individuals with underlying disabilities such as heart problems, asthma, chronic lung disease and cancer are more vulnerable to severe disease. These peoples have to take more precaution to prevent covid-19 infections.

Ethical Clearance- Not required

Source of funding- No funding

Conflict of Interest - Nil

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Efficacy of Manual and Digital Contact tracing of COVID-19

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Abstract

Background: The unprecedented situation unfolded by the COVID-19 pandemic needs tools and measures of varying degrees. Contact tracing is one of them. Contact tracing has become buzz word these days. Along with non-pharmacological interventions (NPI) this is one of the major tools employed by the local health care authorities.

Summary: The contact tracing measure is major part of the response against COVID-19 pandemic. Methods of contact tracing and its efficient way to carry them out needs to be studied. The efficacy of the contact tracing mechanism needs strong backing of empirical data upon which research is ongoing. Further combining the manual and digital contact tracing is the option authorities needs to check out. Health care human resource crunch can be addressed by creating civilian vigilantes to inform the health authorities about the possible suspected case. In case of digital tracing the data minimization and anonymizing the data in which there is no name attached with the particular data can used so that doubts about data privacy should get vanished. Balancing between controlling case fatality rate and infection rate with the help of proper and adequate resourceful data is the key to effectively control the spread along with easing of restriction and step towards normalization of lives.

Conclusion: More thorough study is needed with numerical data backing to establish the efficacy and the relation between controlling the case count and contact tracing.

Key Words: - Contact Tracing, Covid-19, Infodemic, Primary Tracing, Secondary Tracing, High Risk

Introduction

The coronavirus disease 2019 shortly named as COVID-19 is wreaking havoc in all parts of the world. Started from the Hubei province of Wuhan in Peoples Republic OF China, the spread and reach of the virus has reached every nook and corner of the world. Nearly 1 million deaths and 32 million cases worldwide it effectively put pressure on already crumbling health infrastructure. Named after its shape of crown the coronavirus is hitting harder and like never before to all the humanity. As vaccine is under trial and will need some more time and cannot be expected in near future, it is essential to innovate various methods to be employed in the containment of the deadly virus which has been employed and being rectified according to the feedback. Initially the ambiguity of whether this disease spread through human to human contact or not was there but later World Health Organization (WHO) clarified that this can be transmitted from human to human. This triggered the need of identifying the persons carrying the load of coronavirus. First the authorities tried to treat them but later found out that before treatment the infected person might have contacted with other peoples steadily increasing the patients count. Then the need of contact tracing was really felt and authorities started to chalk out the plan to contain the spread of the deadly disease. The test, trace and treat model immensely helped in containing the cases during early period. Initially only family members were isolated and quarantined but on the course of the time other immediate contacts such as neighbors and house help also got isolated. This prove out to be a great strategy for containment as where resources are lacking and where there is low doctor to population ratio. Densely populated countries where health infrastructure is already burdened now experiencing the crushing effect of added pandemic. In such situation this strategy of policy making to contain the spread is
really helping to avoid the unfortunate scenario. As the test, trace, treat model evolves, testing eventually becomes an essential part in success of the contact tracing methodology. Proper rules and regulations and training should be undertaken by swab takers which are in turn health care professionals to properly take the swab which can correctly decide the infection level.\(^{(3)}\)

Efficacy of the contact tracing is also under scanner as more study is needed to establish the correlation between number of cases and tools employed. Which should be employed and where is a crucial factor in containing the viral spread. Various methods according to the ground reality are being employed and rectified every day to get on the nerves of this unprecedented situation. The role of technology is also crucial in contact tracing in particular as the penetration of the network of the cellular phones with internet is ever increasing and on the level that was not seen before. Also, ethical dilemmas such as right to privacy should be given thought while formulating any guidelines on the digital contact tracing. This article unfolds some aspects involving the contact tracing strategy to effectively contain the viral spread.\(^{(4)}\)

**What is contact tracing?**

Contact tracing has become buzz word these days. People who were in close contact of a person whose report is positive for contagious virus such as coronavirus and are at higher risk of becoming infected with the said infection and who has potential to further infect others are called close contacts of the infected person. Tracing and isolating such people if necessary are known as contact tracing. Contact tracing becomes extremely important in such scenario where spreading rate of contagious virus is high such as novel coronavirus.\(^{(5)}\)

Contact tracing can be broadly divided into three basic steps. In which first one is contact identification. Once some people confirmed with such virus, such person is asked about his recent activities where the person possibly came in contact with another people. These contacts can be anyone from family members to house keepers. Second steps are called as contact listing. All the persons listed by the above infected person should be listed as contacts. It can be further classified into high risk category and low risk category. As receiving early care is of utmost importance, these contacts should be identified and conveyed the necessary message and acquaint them of further procedure. The third and the last step in the contact tracing procedure is called as contact follow up. Regular and in appropriate time the contacts should be checked and monitored by the health authorities for symptoms. Asymptomatic contacts can be kept at home with proper medical interventions. But even mild symptoms contact should be admitted to the hospital as soon as possible otherwise his health would worsen and risk of transmitting the virus is maximized which is low in the asymptomatic cases hence quarantined at home.\(^{(6)}\)

Further contacts can be further divided into primary and secondary contact. Primary contacts are those who were within close proximity of six feet for at least two days prior of the confirmed positive report. These can also call as close contact. Secondary contacts are those contacts whose proximity with the confirmed positive patient were less than the primary contact. In case of lack of healthcare infrastructure and overwhelming scenario secondary contacts can be quarantined at home with appropriate care provided they are asymptomatic and having less comorbidities. While primary contacts should be monitored closely and hospitalized even if minor symptoms are shown. High risk contacts are those contacts whose age is above 50 years and having different comorbidities. Research showed that the virus affects more comorbid patients than healthy ones.\(^{(7)}\)

**Methods of contact tracing**

World Health Organization has issued guidelines that must be adopted with necessary changes to effectively counter the viral spread. Depending on the epidemiological scenarios there are some case by case basis response level. For example, what should one do when there are zero cases, when there are sporadic cases here and there, when there is cluster outbreak and finally when there is community transmission where one does not know from where he or she got the infection? [WHO INTERIM] In first two scenarios where cases are zero or sporadic occurrences, health infrastructure can be strengthen and necessary medicines can told to manufacture as lot and equipment’s should be strengthen. In second and third scenario cluster outbreak, needs intensive contact tracing to avoid upgradation into community transmission. Finally, when community transmission takes place then it is extremely difficult to effectively employ the contact tracing strategy especially in the countries with huge population and population density. It is hard to completely defenestrate the virus at
one go but due diligence is essentially required. Contact tracing can reduce the further infection by up to 15 percent which is remarkable feat.(8) Self-reporting is also an effective way to counter the disease spread. But that needs proper dissemination of information by authorized agencies. The preference level for isolation of the suspected patient whose report is under progress should also be decided by the authorities. The prime importance should be given to the health care professionals who are the frontline workers in this pandemic then according to the comorbidities and risk factor associated the next in line should be decided according to the ground condition. The Pandemic also parallel accompanied by Infodemic which is lot deadlier than previous. In some cases it even prevented from reporting the patient by creating a hoax.(1) As the person having the infection is not able to disclose its identity publicly which would have been a best option, due to attached taboo, the healthcare professionals manually trace the nearest past contact of the infected patients. People’s participation is extremely necessary in this complicated task. The right information would eventually bring down the casualties due to late reporting of the cases.(9) Efficiency of contact tracing Contact tracing is the main response on which local health authorities can rely upon to control the spread. An average individual has on an average has 36 contacts and among all people around 9 percent of them have around 100 contacts.(11) To sharpen the contact tracing strategy maximum number of secondary contacts needs to be traced so that they are identified before they get infected and spreader. Longer time would eventually increase the case count day by day exponentially. All the contacts need to be monitored as not all the contacts are symptomatic and will render test negative. They possible can became infectious after some days. The efficacy of the contact tracing is also depending on the nature of primary and secondary contacts. The comorbidity plays a very vital role in case fatality rate (CFR). The more medical conditions one is suffering from more is the chance of one to be turn out a severe case. As more and more governments are turning towards easing up the social restrictions norms, their strategy focuses on reducing the case fatality rate (CFR) than reducing the number of infections. So, it would be disaster if more comorbid which are also known as high risk patients or contacts exposed to the virus. There is a need of correcting the definition and criteria of close contact and secondary contacts. Almost every government have their different perception of close contact. Contact tracing can go hand in hand with other non-pharmacological interventions (NPI) like physical distancing, wearing of masks, and sanitization on regular intervals to lessen the
burden of the health authorities. Apart from what is being discussed the major tool for the local health authorities is definitely the contact tracing strategy. It is efficient so far and its importance is increasing as the case count is surging on daily basis.\textsuperscript{(12,13)}

\textbf{A case for digitally tracing the high-risk contact}

Authorities are relying on the manual tracing of the contacts of the detected COVID-19 patients. As the exponentially of the pandemic is ever increasing it is necessary for the authorities to trace as soon as possible. Taking the help of the technologies will surely help as we experiencing technological revolution. The penetration of the internet connectivity will only help in tracing the contacts.\textsuperscript{(14)} In India the government has launched Arogya setu application which can be downloaded on the cellular phones of the peoples. It uses Bluetooth as well as location data to alert the folks about nearby positive cases so that it can secure itself from that particular area. Further it is also helpful in crowded places such as transport and various congregations where large gatherings are expected. Close circuit television camera’s (CCTV) can be used with thermal gun to monitor the temperature and isolate the suspected patients. It is far rapid than the manual method where the chance of the infection of health care processional is also high. The confirmed positive report of a patient should automatically trigger the authorities to trace its digital contact in near past and alert them as they also can be potent infection spreader.\textsuperscript{(15)} These days cellular phones popularly known as mobile is the nearest possible thing of a person so further process becomes easy. The asymptomatic patients can be isolated under supervision in their respective homes as chances of early detection reduces the spreading capacity of a patient. There is a direct relation between population adopting digital contact tracing application and decrease in number of infections. Both are in direct proportion. Means as the download and usage count of the tracing application increases it further facilitates in identifying the contacts rapidly and quarantine or isolate them further to reduce their exposure to the masses. In country like India where there is already lack of manpower and infrastructure this methodology will immensely help in getting off some of the health professionals’ burden. Even if it covers small number of our population it is very rapid and can further prevent the spread by identifying the risky contacts rapidly. The pandemic already wreaking havoc and any possible and innovative solution should be tried to check its feasibility. But the efficiency of the application-based contact tracing increases with the large cross section of the population uses it. Otherwise it will render ineffective tool. Also users should use the application in a continuous manner then and only then it will show some intended results.\textsuperscript{(15)}

But the other side of the coin also has strong point to make. As this pandemic unfolds the power wielding nature of the various governmental authorities, handing over information of citizens which they are hesitate to share is also a big concern. Too much information at the disposal of the authorities can crush the opposition’s voice. WHO In its interim guidelines suggested for rational usage of the technology in digitally contact tracing. It suggested measures like data minimization, limited retention of data, independent oversight, and accountability to be in regular use while employing the digital tracing method.\textsuperscript{(15)} In addition, the non-state actors can unethically exploit the loopholes in the governmental data storage and possible leakage of the information such as whereabouts of the peoples is also detrimental to the overall wellbeing of the people. Further proper usage from the user’s side is also necessary as selective usage will only create false sense of security.\textsuperscript{(4)}

A wider and quick consultation with all the stake holders about the digital tracing and proper, unambiguous guidelines should be issued by the authorities. Anonymizing the data is one way out as it will not disclose the person’s identity and will also serve the purpose of faster tracing. Using technology is the need of the hour but concerns of the people are also legitimate. Judicious and ethical usage is the only way out to conquer the unleashed dragon like COVID-19 pandemic.\textsuperscript{(16)}

\textbf{Conclusion}

As there is rapid surge in COVID-19 cases worldwide, almost 32 million cases registered so far, various schemes are being chalked out and tested continuously. Contact tracing is one of the efficient and reliable method to counter the spread of the virus. Definition and criteria of the contact tracing needs periodic rectification. High risk patients or patients having co-morbidities should be
on top in priority list. Protocol on asymptomatic patient as they are undetected and possible spreaders should be monitored regularly. As far as manual tracing is concerned it can be supplemented with digital tracing which is more rapid and the fast. Government can try out hybrid model engaging both, manual as well as digital tracing, to make policies regarding the containment of COVID-19. As governments are turning towards lowering case fatality rate than lowering the number of infections a delicate balance should be established as most of the governments lack resources even after dedicating sizable amount of resources toward dealing with COVID-19 pandemic. Tracing should cover large spectrum of the population and the testing result delivery should be as fast as possible to avoid more spreading. Lack of human resource in health care system is road block in contact tracing like strategies. Self-reporting by people on their own after creating awareness could be effective. Efficacy of the scheme of contact tracing needs more empirical study to prove its profitability. It can act as guiding light in upcoming such unfortunate scenarios. Study on spreading capacity of seconaey patients must be studied as it will also adds up to the containment of the pandemic. Infodemic is also parallely spiraling upward as technology can be misused too. Hesitancy in testing by people because of fake messages world end up creating a whole new disaster. Case for digital tracing is strong as these unprecedented situations requires utmost solutions. Technical glitches are hindrance in employing the application-based tracing which can be removed by strengthening the back-end support system of the application. Although manual tracing is rendering more result the digital one is faster. Limited reach of the technology is a cause of concern in employing it. Proper guidelines regarding how to use the application should be popularize thorough various platforms so that the masses can use them with ease. But also, the ethical concerns of the users about privacy and data usage should be addressed so that we can effectively use the technological advancements.

**Ethical Approval:** IEC, DMIMS, Wardha

**Conflict of Interest:** Nil

**Funding:** DMIMS, Wardha

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Survey on Parental Attitude Towards Ethical Considerations of Involving their Children in Physiotherapy Care

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Abstract

BACKGROUND: Ethical considerations around Physiotherapy treatment on Children are more complex than discussions about adult involvement. The interrelation between the ethical values and its involvement in practice is a challenging one. The accountability of clinical decision making by the therapists differ for children and adults. The implications of ethical values in practice provide quality good care for the patient. The purpose of this study was to explore the parental attitudes towards ethical considerations of involving their children in Physiotherapy care.

METHODS: Total of 38 parents whose children undergoing Physiotherapy session at present were participated in the survey. Participants were chosen using convenient sampling technique. The instrument used in the study was a self-descriptive questionnaire. The data were analysed using descriptive statistics.

CONCLUSION: The result revealed that there is no marked evidence of ethical misconduct seen among Physiotherapists handling the Children and it was found that there is a bias in some of the ethical norms like getting written informed consent before treatment, documentation of the records. Efforts to focus on the elements which has a bias and if rectified will have a positive effect on quality Physiotherapy treatment for the children.

Keywords: Ethical considerations, Parental attitude, Parental care, Physiotherapy care, Quality of treatment.

Introduction

Quality of care combines both the care provided as well as the criteria for what constitutes good care. Patient satisfaction is an indicator of quality of care (¹). Number of studies is been evolving which says about patient satisfaction with the medical care of their children (²,⁴,¹⁶).

Every child is special to a parent. Parental care and treatment services are determined based on the special needs of some children (³). In the Physiotherapy care for children, Physiotherapists normally view Children and their families in intervention services and treatment plan and set goals to provide the Child with better quality of life to be best extent and with more opportunities to acquire motor abilities and longevity.

Ethical considerations around Physiotherapy treatment on Children are more complex than discussions about adult involvement (⁵).

Children are the highly susceptible population as they depend on their parents or caregivers for care and protection. The liability for autonomy in decision making for this group of population is always influenced by their authority figures (⁷).

Professional competencies which include ethical knowledge, ethical problems and the skills to handle the problems play a major role and are much needed in the modern healthcare area. To develop the betterment of quality of care for the child, there is a need to determine how widespread the use of ethical guidelines in the practice of Physiotherapy was.

The purpose of this study was to rule out the parental attitude towards the use of ethical guidelines in Physiotherapy session where their Children are being rehabilitated.

As parental care is an important aspect in the Physiotherapy rehabilitation, their attitude towards the knowledge of ethical guidelines to be followed
by the physiotherapists handling their child must be encountered. This set the base to know how quality Physiotherapy is been achieved for their child.

**Materials and Methods**

This was a study conducted in order to explore the attitude of parents towards quality treatment taken by their children in Physiotherapy care. This study was carried out in two rehabilitation centers in Chennai, Tamil nadu. Participants were selected through convenient sampling. Questionnaires were administered to 38 parents who had brought their children for Physiotherapy treatment. There were four non respondents. Data analyses were therefore based on 38 questionnaires.

The questionnaire used in this study was a self-descriptive questionnaire for which content validity and reliability was taken.

1. Demographic data survey instrument:

The demographic data form consists of items to elicit information regarding age, gender, education and presence of the children undergoing Physiotherapy at present. Also it consists of details of parents.

2. Questionnaire:

The questionnaire comprised of a 19 questions of a likert-type scale which serves as a measuring tool for this study. This questionnaire was developed using statements from ethical principles stated by World Confederation of Physical therapy. Questions were designed to provide information on parent attitude towards quality Physiotherapy care for their children, respect, level of remuneration, etc. parents has to indicate to which degree they agree (or not) with each statement by encircling the statement corresponding to one of four statement categories varying from “strongly agree” to “strongly disagree”. Content validity was found for the questionnaire and has been established. It was found out through examination of the questionnaire by health care staffs.

Data was collected by explaining briefly about the aims and method of the study to all the parents participating in the study. Parents who were willing to participate were asked to complete the questionnaire. Table 1 shows the demographic data of the parents who participated in the study and it also shows the characteristics of the children who are involved in physiotherapy care. The time taken to complete the questionnaire was 15 to 20 minutes. Participants were assured that the information given by them will be protected and remain confidential. Study participant was chosen as parents to complete the questionnaire since in the pyramid of Child rehabilitation, Physiotherapist and the parent accompanying the Child, only the parent can be able provide the absolute and correct information on how ethically was the treatment carrying during the session. And the misconducts in the Physiotherapy session can also be identified from the data obtained from the parents.

<table>
<thead>
<tr>
<th>Table 1: Demographic data</th>
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</thead>
<tbody>
<tr>
<td>Characteristics of participants participated in the study</td>
</tr>
<tr>
<td>Parents</td>
</tr>
<tr>
<td>Female gender (Mother who participated in the study)</td>
</tr>
<tr>
<td>Male gender (Father who participated in the study)</td>
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</tbody>
</table>
Results and Discussion

The result of this study shows that, according to parent’s attitude, there is no marked evidence of ethical misconduct seen among Physiotherapist during the Physiotherapy session. From the data available, it was shown that the physiotherapists handling the Children are highly following all the ethical guidelines except some one or two considerations. As per the guidelines given by World Confederation for Physical therapy (WCPT), it expects Physiotherapists to Respect the rights and dignity of all individual, the physical therapist should act in accordance with the laws and regulations ruling the practice of physical therapy in the country in which they practice, Physical therapists must have a knowledge to make independent judgement, Physical therapist should provide honest, competent and quality services, physical therapist must give a legal right to a just and fair level of remuneration for their services, Physical therapists must provide accurate information to patients/clients about physical therapy treatment. Based on these ethical principles, the questionnaire was made and data were collected from the parents.

Table 2: Parent’s response to the items or domains in the questionnaire (Number of respondents n=34)

<table>
<thead>
<tr>
<th>S.NO</th>
<th>ITEMS/DOMAINS IN THE QUESTIONNAIRE WHICH ASKS QUESTIONS TO THE PARENTS TO KNOW THEIR ATTITUDE ON.</th>
<th>NEGATIVE RESPONSE Rating of 1 &amp; 2</th>
<th>POSITIVE RESPONSE Rating of 3 &amp; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Satisfactory physiotherapy care given to the child</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>receiving sufficient information concerning child’s condition/course of illness</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>understanding the information they received about their child’s condition</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>Probability of getting opportunity to discuss the goals of child’s treatment with the physiotherapist</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>Probability of getting opportunity to participate in discussions concerning their child’s treatment</td>
<td>1</td>
<td>33</td>
</tr>
</tbody>
</table>
The main aim of this study is to find out how quality physiotherapy treatment is given to the child involved in physiotherapy care. The connection between the ethical values and its involvement in practice is a challenging one and every physiotherapist must pay attention to the implication of ethics in their practice to provide quality good care. This saying goes in hand with one study by Kati kulju et al, where the authors in that study found the ethical problems faced by the therapist in their practice and there is a state that with moral conduct and moral sensitivity by the physiotherapist, the quality good care can be achieved (11).

Cont.. Table 2: Parent’s response to the items or domains in the questionnaire (Number of respondents n=34)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>Possibility to ask questions about their child’s condition</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>Written informed consent</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>Respect for the child by the physiotherapist during physiotherapy session</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>9</td>
<td>Have you been asked about your opinion on satisfaction about physiotherapy service for your child</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>10</td>
<td>Probability of understanding the nature of the physiotherapy service being provided especially on time and financial basis</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>11</td>
<td>physiotherapy care with regards to the cooperation by the physiotherapist</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>12</td>
<td>Opinion on efficient physiotherapy care given to their child</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>13</td>
<td>Opinion on whether physiotherapist handling their child work towards goal – good care for their child</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>14</td>
<td>Opinion on Physiotherapist maintaining adequate records to allow for the evaluation of the child’s care</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>15</td>
<td>Confidentiality about child’s information</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>16</td>
<td>Opinion on how physiotherapist who handle their child allow their service in a proper way</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>17</td>
<td>Child receiving satisfactory treatment within in a reasonable period of time</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>18</td>
<td>Confidence in their physiotherapist skill</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>19</td>
<td>Opinion on physiotherapist’s responsiveness to their child’s needs/requests</td>
<td>0</td>
<td>34</td>
</tr>
</tbody>
</table>
The professional relationship between therapist and patient plays a major role to carry out goal directed treatment approach where both the need of the patient and the goal of the therapists should be achieved without any conflicts. In a study by Andrew A. Guiccione stated and found this issue of conflicts arising in professional practice with regards to professional relationship between therapist and patient (9). In case of paediatric treatment approach, the goal directed treatment by the therapist is as important as achieving the needs of the patient (both parent and child). This conflict can be solved out when the therapist handling children educate the parents about the diagnosis and prognosis of the child and if the treatment goal is carried out by giving opportunity to the parents to tell their opinion regarding their needs for their children. This study focused these issues and questions were asked to the parents accordingly. It shows positive response that physiotherapists handling the children are educating the parents about their child’s condition and prognosis and they are following a goal directed treatment by keeping parents view on the need for the child.

From the obtained data (Table 2), there was a positive response from the parents for most ethical considerations like respecting the Child’s needs, about satisfactory Physiotherapy care based on the money paid and time spent. There was 97.1% positive response towards domains like confidentiality, setting the treatment goals with parents. 94.1% of positive response was found for parent’s opportunity to participate in discussions concerning Child’s condition and treatment. Also, it was found that there is a bias only in the domain like maintaining documentation of records which showed 88.2% of positive response and getting written informed consent which only showed 55.9% of positive response. Getting written informed consent should be followed by physiotherapist handling children before starting the treatment and it will have a positive impact and confidence in the therapist skills by the parents and it also ensures therapists safety and precautious measures before handling the patient. Next point to focus is, documentation of records in the Physiotherapy session should be an important one which should be focused and followed in future as that would provide the information of prognosis of the child.

Conclusion

The study shows that the physiotherapists handling the children are highly following all the ethical guidelines during the Physiotherapy session. Hence the study concludes that there is no marked evidence of ethical misconduct seen among the physiotherapists handling the children in Physiotherapy care. In order to make betterment in the quality of Physiotherapy care for children and for the development of the profession, the practicing physiotherapists must follow all the ethical guidelines in their practice.

Acknowledgment: A hearty appreciation and thanks to all those parents who accepted to participate in this study.

Ethical Clearance: Departmental ethical committee clearance was obtained before conducting the study.

Conflict of Interest: Nil

Source of Funding: Self

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A Rare Case Series of Prosthetic Rehabilitation of Auricular Defect Category: Case Series

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Abstract

Loss of facial organs in an individual may be developmental anomalies or acquired. The missing parts of the face ear, eyes and nose are considered as maxillofacial defects which can be rehabilitated by the prosthesis and/or cosmetic surgeries. This art of science has developed into a more reliable and predictable process due to ever increasing development of materials and equipment used in the procedure. This article describes a simple technique to rehabilitate patients with auricular defects which are both aesthetically acceptable and economical for the individual.

Keywords: Maxillofacial defects, Auricular prosthesis, Facial rehabilitation, Spectacle retention

“Disfigurement is the sign telling the world that you have survived from what tried to kill you, well they will tell you all those sorts of solace, deep down humans are still run by societal laws and these laws are brutal they coerce you into adapting into their image of superficial beauty which rather controls your own understanding of self-esteem, so until the time the society acts on what it speaks we embrace the fact that satisfaction lies not in content but validation”

Introduction:

“The love of life is next to the love of our own face. And thus the mutilated cry for help.”

Sushruta Samhita, India, 600 B.C.

The face is an extremely important symbol of man although not the only one. Facial deformities are concealed with great difficulty and jeopardize the appearance of a person more seriously than do similar defects in other parts of the body. Because of this fact they are the most common disfigurements which produce psychic distress. A slight facial deformity frequently produces a psychological effect out of all proportion to the extent or appearance of the disfigurement¹. Microtia is quite often categorised as congenital deformity where the external ear is underdeveloped. A completely undeveloped pinna is referred to as Anotia. Because microtia and anotia have the same origin, it can be referred to as Microtia-Anotia². Microtia can be unilateral (one side only) or bilateral (affecting both sides). Microtia occurs in 1 out of about 8,000–10,000 births. In unilateral microtia, the right ear is most commonly affected.

The aetiology of microtia remains undefined but there are few cases that associate the cause with genetic disorders or with gestational diabetes³. Genetic inheritance has not been fully studied but in the few studies available, it has shown to occur during the early stages of pregnancy³.

Despite remarkable advances in surgical management of oral and maxillofacial defects, many such defects, especially those involving eyes and ears cannot be satisfactorily repaired by plastic surgery alone. Maxillofacial Prosthodontists are highly skilled prosthodontist who have advanced training in complex oral rehabilitation. They carry these treatments of managing these facial defects or other in a multidisciplinary team comprising of maxillofacial surgeons, ENT surgeons, Plastic surgeons, neurosurgeons, speech therapists and clinical oncologists. The principle being enhancement of quality of life of the patient.
According to Chalian (1970) objectives of maxillofacial prosthetics include, 1) Restoration of aesthetic appearance, function 2) Protection of tissue 3) Therapeutic healing affects 4) Healing the Psyche. Prostheses are made to replace hard tissues like bone and teeth, or soft tissue to restore oral function and form. Prosthetics also include devices which protect and shield the soft tissue from radiation during the procedure. On account of all the services that a prosthodontist provides, maxillofacial Prosthodontists (MFP) are trained in working with a multidisciplinary setting together with maxillofacial surgeons; otolaryngologists; cosmetic surgeons; speech pathologists; etc. The rationale stated above clearly qualifies the maxillofacial prosthodontist to rehabilitate deformities. Maxillofacial prosthetic rehabilitation is a justified alternative when surgical reconstruction is not feasible or desirable. The success of extra-oral prosthesis are dependent on the modes of retention, which may be using biological adhesive, utilization of undercuts, retention by other customized devices (e.g- spectacles), magnets, endosseous implants. The following case series is regarding rehabilitation of lost ear with maxillofacial prosthetics which are retained by various modes of retention, providing an insight into the options the prostheticians have.

Case Series:

The artificial ear must be a mirror image of the natural ear, the impression of the natural ear must be made along with that of the defect to fulfil this criterion. Guide lines must also be drawn on the face of the patient to establish proper positioning of the prosthesis coinciding with the natural ear, also anatomical landmarks can be used as a stable guide. A set of three cases will be discussed simultaneously under the general headings of steps to be followed for fabrication. Informed written consent for case recording and photographs were obtained from the patient before the beginning of the procedure.

Step 1: Case evaluation and Planning (Figure 1)

Figure 1 A, B: Preoperative photograph of the patient with missing ear defect.

Figure 1 C-Preoperative photograph with right ear missing; D- Endo-osseous implants placed as a retentive aid planned for the prosthesis.

Figure 1 E; preoperative photograph of the defect area; F- 2 implants were placed in the temporal region for bar attachment as a retentive aid planned for the prosthesis.

Case 1:

A 28-year-old Female Reported to the department of Prosthodontics and maxillofacial prosthetics, with a chief compliant of missing ear (Figure 1 A,B). On careful history recording and clinical examination it was deduced that the patient had a congenitally missing ear with a rudimentary lobe which consisted of cartilage. After all the option were explained to the patient, the patient opted for an auricular prosthesis which would be supported by customized silver attachments.

Case 2:

A 19-year-old male reported to the department of prosthodontics and maxillofacial prosthetics with a chief complaint of missing ear. The patient had a congenitally missing ear with the external auditory meatus opening near the angle of the mandible on the right side (Figure 1 C). Implant supported auricular prosthesis was planned keeping in view the young age of the patient. Using guide lines the new position of the ear was determined coinciding the natural ear. After radiographic examination
the positions of the implants were decided. Three 4mm EO implants (Straumann, AG, and Switzerland) were placed in the temporal region (Figure 1 D). After Osseo integration and healing, ball and socket attachments were placed before impression making. The prosthesis was to be supported by a customized acrylic scaffold which would retain on the implant by means of socket type attachments.

Case 3:

A 33-year-old female reported to the department of prosthodontics and maxillofacial prosthetics with a chief complain of missing ear(Figure 1 E). The ear was congenitally absent with the external auditory meatus opening near the posterior border of the mandible. An implant supported prosthesis was planned after radiographic examination of the prospective site of the ear. Two 4mm EO implants (Straumann, AG, Switzerland) were placed in the temporal region. Castable abutments were used and were connected using inlay wax, later the entire assembly was casted and checked for fit over the implant. The prosthesis was to be retained on the implant with the help of bar-sleeve attachments connecting the two implants(Figure 1 F). The sleeves were embedded in an acrylic frame work to assist the retention.

Step 2: Impression Making:

The primary goal of impression is to record the defect site with maximum peripheral tissue coverage, if it is possible a facial moulage must be taken as it helps in reference for ease of sculpting for mirror image of the natural ear. Impression of a suitable donor ear is also taken, if donor ear is not available. The decision of the retention areas are then made whether to choose implant supported or mechanically retained prosthesis. For implant supported prosthesis, impressions were recorded after placement of implants and the abutments. The ear canals are blocked with cotton and petroleum jelly is applied around the hairy areas. The area to be recorded is boxed using cardboard scaffold. Irreversible hydrocolloid material (Zelgan, Dentsply) was used in a thin consistency and poured in the area, the impression was reinforced by pouring a layer of fast setting plaster on the impression. After careful inspection of the impression it was poured in dental stone (kalstone, kalabhai)

Modes of Retention:

The Methods of Retention and their peculiarities are presented in table 1 with photographs below:

Figure 3-(Case 1), A-illustrates the customised silver struts and wire attachments used as a retentive aid; Acrylic mock trail substructure for evaluation of the efficacy of the retentive aids used. Definitive silicone prosthesis attached through the struts and customised silver wires as retentive aid. 

Figure 3-(Case 2), B- Acrylic substructure over the attachment component; (Ball and socket type of attachment)
Figure 3-(Case 3), C- depicts the Bar attachment casted in cobalt-chrome; b illustrates the sleeves attached onto the bar used as a retentive aid for prosthesis; c acrylic substructure housing the sleeves of the bar and sleeve attachment assembly.

**TABLE 1: Methods of Retention used in the cases.**

<table>
<thead>
<tr>
<th>RETENTION</th>
<th>Case 1 Figure 3, A</th>
<th>Case 2 Figure 3, B</th>
<th>Case 3 Figure 3, C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Retention</td>
<td>Mechanical, soft tissue based</td>
<td>Mechanical Implant supported</td>
<td>Mechanical implant supported</td>
</tr>
<tr>
<td>Form of assembly for retention</td>
<td>Customized silver attachments</td>
<td>Ball and socket type of attachment.</td>
<td>Bar and sleeve type of attachment casted in cobalt-chrome</td>
</tr>
<tr>
<td>Surgical Procedure required</td>
<td>Minor surgery to establish channels in cartilage for placement of silver struts</td>
<td>Implant placement Three 4 mm EO implants (Straumann, AG, Switzerland)</td>
<td>Implant Placement Two 4 mm EO implants (Straumann, AG, Switzerland)</td>
</tr>
<tr>
<td>Period post-surgery</td>
<td>After 3 weeks post-epithelization of the channels</td>
<td>Post soft tissue healing and osseointegration (3-months)</td>
<td>Post soft tissue healing and osseointegration (3-months)</td>
</tr>
<tr>
<td>Use of substructure</td>
<td>NA</td>
<td>Acrylic substructure over the attachment component</td>
<td>Acrylic substructure incorporating sleeves</td>
</tr>
<tr>
<td>Method of fabrication of substructure</td>
<td>NA</td>
<td>Acrylization of wax pattern over the models post abutment and attachment placement</td>
<td>Acrylization of wax pattern over the models post abutment and bar assembly placement with sleeves</td>
</tr>
</tbody>
</table>

**Step 3: Wax Pattern Fabrication and Trial**

Trial pattern can be fabricated using modelling clay or wax. Modelling waxes are preferred. A wax pattern was carved by using the remaining natural ear as reference using modelling wax. A wax trial was performed to ascertain the appearance of the ear. For case 1 two wax pattern trials were made to mark areas of the remaining lobe for making channels for the silver struts and check for the ability of the customized retainers to hold the prosthesis in place. In Case 2 & 3 the acrylic substructure is fabricated with wax and fabricated separately with attachments embedded in them. Wax patterns were retained on these substructures using boxing wax during wax trial.
Step 4: Flasking, Coloration, Fabrication & Finishing of Prosthesis

Fabrication of mould for auricular prosthesis differs from fabrication of prosthesis for other defects. A three-piece mould is fabricated using dental stone (kalstone, kalabhai) and plaster (Neelkanth plaster) for packing of silicones. Shade matching is done and intrinsic stains (Functional Intrinsic II) are added to the room temperature vulcanizing silicone (RTV, Technovent). The mix is thoroughly spatulated to uniformity and packed into the moulds. After 12 hours of setting time, the prosthesis is retrieved. The excess is trimmed and the irregularities are grinded with a fine acrylic bur. The prosthesis is cleaned and a trial is carried out to check the fit of the prosthesis. Extrinsic shading (Technovent Extrinsic colours) is applied by dissolving shades in the RTV-silicone, the prosthesis is left to dry.

Step 5: Prosthesis Delivery

Case 1: (Figure 5 A)

Three holes were made corresponding to the ones made in the trail appointment in the prosthesis and the silver struts were inserted and closed through these holes, the rest of the margins were adapted to the adjacent skin and biological adhesive was used to blend the prosthesis with the surrounding skin.

Case 2 & 3: (Figure 5 B,C)

The acrylic substructure was glued to the prosthesis using cyanoacrylate adhesive and adapted on the implant.
Step 6: Prosthesis Post-Operative Care

The patients were taught how to handle the prosthesis and how to wear it. Use of mild soap was advised to clean the prosthesis. Instructions regarding follow-up and shelf life of the prosthesis were given to the patient.

Discussion

Cosmetic re-construction is one sure way to restore the original tissue, but many times the facial defects are extensive and surgery does not yield good result. Maxillofacial prosthesis is an effective and economic way of rehabilitating facial defects. Maxillofacial silicone is an aesthetic material which can be coloured or stained according to the complexion of the patient, it is easy to manipulate and has a close-to-natural texture. Intrinsic colouration is more stable than extrinsic but they are un-aesthetic as they are homogenous, humans have skin tone differences due to thickness of mucosa, vascularity, presence of cartilage. These are the rationales on which the decision to use RTV-Silicone and extrinsic coloration are based.

Facial defects cripple the patient in a psychologically more than any other aspect. The acceptance of the prosthesis plays a major role. Retention provides psychological comfort to the patient and thus by relation is one of the most important necessity. Literature has discussed numerous ways to retain extra-oral prosthesis.

Adhesives are one of the most popular retentive aid in maxillofacial prosthesis retention. Silicone adhesives are basically RTV-Silicones which are diluted in volatile solvents. On the evaporation of these solvents the set adherent silicone is left which helps in retention. In case 1 there has been use of custom-made silver attachments along with adhesives, the adhesives blend in the thin margins of the prosthesis while the attachments provide the major share of retention. It was a less invasive and economic method for rehabilitation.

Craniofacial implants are used for retention of extra-oral maxillofacial prosthesis as they provide excellent support and retention, this leads up to the improvement in patients appearance and quality of life. According to clinical studies, a minimum of two implants are required to retain an auricular prosthesis.

Ball and socket type of attachment are also effective way of retaining the prosthesis, it is easy for the patient to wear such a prosthesis. the ball and socket type of assembly require the abutments to maintain parallelism so as to facilitate single path of insertion for the prosthesis without shear stress on the implant.

Bar-clip retention is one of the forms of retention used in the auricular region. The limitations of such attachments are that they limit access for cleaning the implant area and patient faces difficulty in insertion and removal of prosthesis. Thus this case series of rare defects found gives us the insight about the multidisciplinary team involved and pivotal role of each member. Role of a Psychiatrist post rehabilitation also contributes to a major extent, the patient is counselled for the same. Rehabilitation of these defects boosts the confidence in the patients and thereby developing a feeling of completeness. Patients become socially active gradually and carry out their routine effectively without denial, hesitation or self-doubt. Thus, the prosthesis not only restores the anatomical defect but also relieves psychological distress.

Conclusion

Facial defects are psychologically traumatising, it is a social stigma. Rehabilitation is not the only treatment which solves these problems. The inflictions of this trauma are deeper than the defects itself. Communication is the key to alleviate this issue. A good quality of life does not stem from treating the problem but helping the patients to recognise that life is beyond the defect. Comprehensive care is deliverable by the dentist as it is him/her who the patient has opened up to.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Clearance: not applicable

Source of Funding: none

References


Prosthetic Rehabilitation of a Patient with Symptoms of Iron Deficiency Anaemia in association with Fraser’s Syndrome – A Case Report

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Abstract

About a quarter of the population in the world is affected with Anaemia and deficiency of iron is one of the major causes. Associated symptoms are long term fatigue, cognitive function which shows impairment, and decreased well-being. “Fraser syndrome” can be described as rare, genetically inherited disorder. It is manifested as Cryptophthalmos, Syndactyly along with Enamel Hypoplasia because of which the patient is referred to a dentist. The following report presents the report of a male patient who had symptoms of iron deficiency anaemia and also showed signs & symptoms of Fraser’s Syndrome. Proper knowledge regarding Fraser’s syndrome and iron deficiency anaemia is imperative to plan an oral rehabilitation treatment protocol for such patients.

Keywords: Iron Deficiency Anaemia, Fraser’s syndrome, Enamel Hypoplasia, Ocular Prosthesis.

Introduction

The main objective for the patients with Iron deficiency Anaemia is facilitation of the understanding and curing measures of the primary causative condition and avoidance of the treatment measures which are invasive and the risks with come along with it.1

The World Health Organization(WHO) has defined iron deficiency anaemia as the one which has haemoglobin values “Less than 7.7 mmol/l (13 g/dl) in men and 7.4 mmol/l (12 g/dl) in women”. Iron deficiency anaemia is characterized by microcytic, hypochromatic erythrocytes and depleted stores of iron in the body. The oral signs and symptoms include sensation of burning of the oral mucosa, lingual varicosity, xerostomia, Oral Lichen Planus, reduced sensory sensations of the oral mucosa and taste dysfunction.2

“Fraser syndrome” was first illustrated in the year of 1962 characterised by Syndactyly, Cryptophthalmos, oral clefting, genitor-urinary malformations, and laryngeal stenosis. The main cause is said to be mutations in “FRAS1”, “FREM2”, or “GRIP1” genes.3

The guidelines for diagnosis of the “Fraser’s syndrome” is given by Thomas et al(1986) which states that the diagnosis meets in those patients which contains at least of two of the major criteria and minimum of one minor criterion or one of the major criterion and four of the minor criteria.4

Table No.1 Diagnostic Criteria for Fraser’s Syndrome4

<table>
<thead>
<tr>
<th>Major Criteria</th>
<th>Minor Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Syndactyly</td>
<td>1 Congenital malformation of ears</td>
</tr>
<tr>
<td>2. Cryptophthalmos.</td>
<td>2 Congenital malformation of nose</td>
</tr>
<tr>
<td>3 Affected Siblings.</td>
<td>3 Cleft lip and/or palate</td>
</tr>
<tr>
<td>4. Abnormal genitalia.</td>
<td>4. Congenital malformation of larynx</td>
</tr>
<tr>
<td></td>
<td>5 Umbilical hernia.</td>
</tr>
<tr>
<td></td>
<td>6. Skeletal defects</td>
</tr>
<tr>
<td></td>
<td>7 Mental retardation.</td>
</tr>
<tr>
<td></td>
<td>8. Renal agenesis</td>
</tr>
</tbody>
</table>

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Oral Manifestations for the syndrome include facial asymmetry, cleft palate along with conditions like Enamel Hypoplasia. Most common signs and symptoms which the patient presents are cryptophthalmos and, syndactyly.  

**Case Report**

A 24 year old Male patient reported to the private dental clinic with the chief complaint of missing teeth, caries and poor aesthetics. He was born second to the consanguineous parents. During his childhood he went multiple surgeries to correct genitalia abnormalities. The patient was born without one kidney and has not undergone any treatment for the same. His intellectual level was normal and no history of any medical disease or disorder was seen in the family.

Ophthalmologic examination showed cryptophthalmos with loss of vision. Head, Face and Neck check up demonstrated hypertelorism which was mild, slight facial-asymmetry and slight reduction in lower facial height. (Figure Nos. 1)

Oral examination indicated microdontia which was generalized in nature and involved dentitions of arches with reduction in their size. He had poorly developed teeth with oral hygiene which was diagnosed with gingivitis and Hypoplasia of the enamel. Patient had reduced taste sensations and burning in oral mucosa which further hinted for diagnostic tests for Iron deficiency anaemia. Teeth present were 11-18, 21-28, 31-37 and 41-47 with grade II mobility seen with 11, 21, 31, 41 and 42. Radiographic examination showed teeth with short roots. (Figure No. 2)

**Management of Anaemia due to Iron Deficiency:**

Blood tests were done to estimate the Haemoglobin level which revealed 11g/dl value. The peripheral smear showed hypochromic, microcytic, erythrocytes and low iron stores. (Figure No. 3)

Invasive tests like anti-parietal cell antibody, celiac serology, Stool sample was tested for H. pylori infection & faecal occult blood test were carried out. Iron replacement therapy was initiated to check the response for a span of 4 to 8 weeks. There was considerable improvement in the haemoglobin level of the patient (14g/dl) and follow up was carried out after 3 to 6 months.

**Dental Rehabilitation:**

To meet the requirements of the patient, a complete tooth supported overdenture was considered. A proper sequential treatment protocol was planned. Teeth with compromised periodontal support were extracted and Elective Endodontics was implemented in the remaining teeth and the preparation was done in a dome shaped contour which could receive copings.

Custom post patterns were fabricated and an impression was made. The copings were finished and polished and luted to the abutment teeth with the help of GIC luting cement (Figure No. 4).

After primary impression, border moulding was done and secondary impression was made. After Jaw Relation and a successful try in, processing was done using heat-cure acrylic resin. The denture was well accepted by the patient and had satisfactory results in terms of aesthetics and function.

**Occular Rehabilitation:**

An acrylic special tray with holes and a syringe tip was made for impression procedure (Figure No. 5). Impression was made using light body elastomeric material. Indentations were marked for proper orientation of the cast which was obtained with respect to fabrication of further wax pattern. The wax pattern was fabricated so as to resemble and simulate the eye which was lost. The stock scleral eye was opted after comparing with the normal eye of the patient. That iris from the stock eye shell was embedded in the pattern. (Figure No. 6).

Flasking and Dewaxing was carried out for the adapted wax pattern. Packing was done with Self-cure tooth colour acrylic Polymerizing resin. After finishing and polishing spectacles were used to make the borders of the ocular prosthesis inconspicuous. Necessary instructions for placement, cleaning, and prosthesis removal were given to the patient. Regular follow up appointments were scheduled (Figure No. 6).
(Figure No. 1 Extraoral Examination of the patient showing reduced lower face height)

(Figure No. 2 Radiographic examination showed teeth with reduced face height)

(Figure No. 3 Peripheral Smear showing Microcytic, Hypochromic anaemia)

(Figure No. 4 The Metal copings Finished, Polished and Luted)

(Figure No. 5 An acrylic special tray with holes and a syringe tip was made for impression procedure)
Discussion

There are several studies which state the importance of immediate and prompt care for the patients with anaemia associated with iron deficiency. It not only eradicates fatigue related symptoms but also elevated the level of quality of life. The treatment of iron deficiency anaemia associated with certain specific syndromes should be undertaken according to the guidelines of the specialist.

The oral supplemental therapy of iron deficiency anaemia may be limited to the patients which do not manifest gastrointestinal signs and symptoms such as cramps in the abdominal region and nausea along with vomiting. Intravenous iron therapy is another alternative for oral iron replacements that is indicated for the patients with blood loss more than 10 ml/ per day.

Fraser’s Syndrome is an autosomal dominant but a rare disorder. Oral manifestations which were met by the patient were as described earlier which included short roots, retained deciduous teeth including Enamel Hypoplasia. After diagnosis of anaemia caused by iron deficiency, identification of the cause should be done or else it will lead to recurrence even after proper treatment protocol.

Cardiovascular abnormalities which are seen in patients with Fraser’s Syndrome include coarctation of aorta, valvular stenosis, ventricular septal defect, atrial septal defect, and cardiomyopathies. If any involvement of cardiovascular system is seen, the protocol regarding infective endocarditis to decrease the microbial load should be carried out. When any sort of Renal Involvement is seen nephrotoxic drugs should be avoided.

Conclusion

Anaemia due Iron deficiency is the major cause of fatigue and impairment in the cognitive function and diminishes the quality of life which is all the aspects of “Health status”, “Lifestyle”, “Life satisfaction”, “Mental health” and “Well-being” reflecting together in an individual. It is an important health outcome and hence Iron deficiency anaemia should be treated upon diagnosis.

Proper knowledge of oral and dental features is definitely going to help with identifying the Fraser Syndrome. Dentistry should reconsider the recent aesthetic approach keeping in account of previous data working its way for successful prosthetic rehabilitation.

Financial Support: None.
Conflict of Interest: None
Ethical Clearance : Obtained

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Increased Demand of Emergency Medical Services in Covid-19

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Abstract

The World is going through a tough situation and that is a pandemic caused by the Novel Corona virus which originated in the Wuhan Province of China in December 2019. During this tough and critical situation, the only saviors of humanity are our health care professionals, who restore the faith and trust in humanity and ensure the safety of each individual suffering from this COVID-19 disease. The cherry on the cake is the additional health support provided by the staff of Emergency Medical Services who help the health care workers in treating the patients in hospital, by giving the pre-requisite or the initial treatment during this emergency situation. So to tackle this pandemic, it is important to meet up to the increasing demand of Emergency Medical Services so that the situation remains under control and there is a decrease in mortality. The fragile and fragmented healthcare system of India needs to be upgraded and emphasis needs to be put on developing the Emergency Medical Services so as to strengthen the healthcare system as a whole and to make living better.

Keywords: fragile, proactive protocol, episodic care, emergency medical services, increased demand

Introduction

No one can deny the fact that the world has changed dramatically in just a matter of few months due to spread of Novel Corona Virus, COVID–19. This COVID-19 is a member of SARS family CoV- 2 Virus and was identified in Wuhan, China in December 2019 and has rapidly spread across the world. India being the second most populous country after China, the presence of this virus in a patient was first detected on 30 January 2020 in Kerala. Being an agronomical (agricultural-based economy) country, the majority of population stays in rural areas and access to health care facilities is difficult. To restrict the spread of the virus in India, a series of lockdown were instituted and as on 8 July 2020 there are 7,46,506 cases and India is ranked 3rd in the world as far as the number of cases are concerned. As the number suggests a diffuse spread of this disease in India, Maharashtra and Tamil Nadu are amongst the worst affected states with both of them nearly contributing around 50% of total cases.

India’s healthcare system is fragile and fragmented. Considering this type of healthcare system during the pandemic caused by Corona Virus, there is immense requirement if Emergency Services at utmost priority.

Emergency Departments are medical treatment facilities which cater to provide episodic care to patients suffering from acute injuries and illness as well as patients who are experiencing sporadic flare ups of underlying chronic medical conditions which require immediate attention. Hence to tackle this pandemic, there is increased demand of Emergency Services and it also highlights the importance of Emergency Medical Services, its education training in a developing country like India.

Reviews

Public health emergencies like pandemic put enormous burden on healthcare systems while revealing deep structural and functional problems in organization care. These difficult times require ones healthcare system to press on acceleration lever and to increase the efficiency of emergency healthcare services and at the same time taking utmost precautions so as to prevent the spread while giving the treatment. (1)

One to this pandemic, there has been an increased need of re-organizing our healthcare system. There has been a complete saturation of healthcare capacities and service. Emergency Medical Services play an important role during this pandemic. (2)
There has been a consistent rise in the number of cases day-by-day. This COVID-19 being an infectious disease, known to be spread by aerosol transmission and affecting mainly the respiratory system of humans, has caused many mortalities especially affecting the older age groups. The increased mortality in older age groups (more than 60 years) is also due to other related comorbidities like Diabetes Mellitus, Hypertension, Bronchial Asthma, Tuberculosis, Immunocompromised patients, cancer patients. Considering these group of patients with underlying comorbidities, most of them are bound to arrive in casualties requiring medical attention at priority in the form of Emergency Medical Services. Emergency Medical Services take into consideration all cases related to any system or organ involved in human body.

During this pandemic caused by COVID-19, the healthcare professionals posted in Emergency Medical Services Department need to wear PPE (Personal Protective Equipment) for their protection. A COVID-19 positive patient with underlying comorbidities may present with attack of asthma, respiratory distress, attack of stroke, Myocardial Ischemia or heart attack, and in such cases immediate medical attention is required. Due to these underlying comorbidities, there have been rise in deaths in India, so somewhere in order to save lives, there is the need of Emergency Medical Services during COVID-19.

To save the lives of these patients EMS professionals have to follow a proactive protocol, just like that followed in a natural disaster “Triage”. There needs to be increase in the amount of medical equipment required in healthcare facilities. Proper PPE (Personal Protective Equipment), ventilators, emergency drugs, ambulances need to be made available and hence their production needs to be increased. These Emergency Medical Services provide urgent pre-hospital treatment and stabilization for severe illness and injuries. It plays an important role in saving life of patients as they carry the patient to healthcare facility and treat them during the transportation time.

So as to enhance the effectiveness of the Emergency Medical Services in India during this pandemic, a proper hotline number should be created so that people have easy access to these Emergency Medical Services as soon as possible. As the spread of COVID-19 is rising exponentially, there should be deployment of ambulances in these zones where there is increase in number of cases, so that during any emergency to a patient, there is easy access to Emergency Medical Services and patient reaches the nearest healthcare facility as soon as possible. As there is increased demand during this pandemic, there should be increased emphasis in training of health professionals in field of Emergency Medical Services. Basic courses like first aid training, Advanced Cardiac Life Support, Basic Life Support, suturing, catheterization should be instituted and made sure health professionals are qualified enough to provide these to patients during emergency. Availability of air-ambulance wherever necessary and required.

As major population of India lives in rural areas, establishment of Emergency Medical Services during COVID-19 pandemic is of utmost importance. Posting of health professionals in rural Primary Health Centre along with availability and access to Telemedicine care will help provide Emergency Healthcare Services to the poor people living in remote areas and help save their life and decrease mortality rate. To make use of the potential of the youth population (especially in the rural areas) and training them to provide basic emergency services during this pandemic will provide aid in decreasing the burden on healthcare professionals. Timely healthcare provided in Emergency Medical Services during this pandemic will help save life of many people and hence justify the statement “Doctors are equal to God” and will help to bring back the faith and trust of people on Doctors. During this pandemic of COVID-19 a major role has been played by Emergency Medical Services efficiently so as to reduce the mortality rate in our country. But as there has been an increased demand of Emergency Medical Services the call volume has explicitly increased on the emergency medical number, thereby leading to unavailability of Emergency Medical Services in remote locations. Hence, a separate Coronavirus Helpline Number should be created so as to streamline the process and help in timely availability of Emergency Medical Services in such localities.

Accessibility and availability of Emergency Medical Services through the use of technology i.e. Tele-Health
for places which are remote and difficult to access. It can be a useful and an efficient option. This portal of technology will help in providing immediate care to patients without the need for any medical professional to go and visit personally. A team of Emergency Medical Services should be made which will cater to needs of people through Tele-Health. (19)

Emergency Medical Services play a major role in trauma and Department of Orthopedics, (20) and during this pandemic there are incidents occurring that have caused disruption and affected the efficiency of Emergency Medical Services. This pandemic is similar to disaster and hence to bring out the maximal (21) output of our Emergency Medical Services is of utmost importance. It is a challenging task to provide adequate Healthcare during this pandemic so there is a need to expand on our Emergency Medical Services so as to make things easier. This Coronavirus outbreak has led us to utilize our resources of medical importance and cater to need of patients and even the Healthcare Professionals. It has led to paying heed to strategizing the need to provide urgent medical healthcare through our Emergency Medical Services. (22)

So as to provide Emergency Medical Services efficiently, the staff working needs to have access to proper N95 respirators, Personal Protective Equipment (PPEs) (23), sanitation practices, and other established safety protocols during COVID-19. Early identification (24) of cases in Emergency Department, which present with symptoms similar to COVID-19, can help restrict the spread of this disease and hence Emergency Medical Services Healthcare Professionals play an important role in this situation of the Coronavirus pandemic across the globe. (25)

**Discussion**

Emergency Medical Services are an integral and important part of health care system and hence are needed during this pandemic. SARS (Severe acute respiratory syndrome)- Corona virus-2, causes COVID-19 disease, is highly contagious and is the causative agent behind this pandemic. (26)

To bring about the effective output of Emergency Medical Services during this pandemic, the fragile and fragmented healthcare system of India needs to form a bridge so as to connect all the aspects of healthcare and to ensure the increased demand. Financial support should be provided by The Government which would be of immense help to meet the rising demand of Emergency Medical Services during this pandemic. (27) The constituents of healthcare which includes Doctors, nurses, Emergency Medical Services staff, ward boys, ambulance drivers and pharmaceutical professionals need to come along together to execute a proactive plan so as to bring about maximum and effective output and help save lives of patients. (28)

Pharmaceutical professionals have important and significant role to play in Emergency Medical Services. Timely availability of emergency medications to the Emergency Medical Services provider staff will help save lives of the patients who are having underlying comorbidities and are also suffering from Corona virus infection. The onus of clinical decision making is less in hands of pharmaceutical professionals in India, so this responsibility of decision making needs to be given to professionals to some extent, so as to bring about rapid response in case of emergencies (29)

Due to this pandemic, there has been an undue pressure on healthcare system of our country and hence so as to release the pressure on the health care system, it is time we require efficient tools for smart governance and resource allocation. To cater this need, we need to stabilize and improve the Emergency Medical Services so that the life of patients is saved. The increased demand of Emergency Medical Services along with their timely action would help to ease out the pressure and decrease the burden on healthcare facility and prevent it’s saturation. (30)

Emergency Medical Services usually include fundamental principles of managing airway, emergency tracheal intubation, cardiac arrest, stroke patients, anesthetic care , Basic Life Support training and hence to meet the increased demand during pandemic, we need to increase the efficiency of the working staff so as to bring about a better output. As a safety measure, prevention is better than cure. So basic precautions like hand hygiene (31): avoiding undue movement and travel (32,33,34) can help a lot to prevent the spread of Covid-19. Also measures need to be taken to reduce depression, anxiety and stress among the general population(35).
Conclusion

During this pandemic, it is of utmost importance to be safe, stay indoors and follow healthy sanitary practices. The improvement in healthcare facilities of our country has led to an additional support in treating these patients suffering from COVID-19. The need of the hour is increased qualitative and quantitative efficacy of Emergency Medical Services so as to meet the increasing demand and also put an emphasis on the development and improvement of Emergency Medical Services so as to help tackle any situation in near future with great ease.

Conflict of Interest: None

Ethical Approval: From IEC, DMIMS, Wardha.

Funding: DMIMS, Wardha

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22. Modelling resource requirements and physician staffing to provide virtual urgent medical care for residents of long-term care homes: a cross-sectional study [Internet]. [cited 2020 Sep 14]. Available from: http://cmajopen.ca/content/8/3/E514.long
Effect of Fluoride Contaminated Groundwater on Human Health in Fluorosis Endemic Areas

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Abstract

Background: Fluorosis is an endemic health problem in twenty-five nations around the globe. Objectives: To correlate fluoride content of groundwater and health status of affected population and suggest remedial measures. Methodology: A cross-sectional research design is followed because people from different age groups were sampled, observations were made and skeletal examinations were conducted at a specified point of time. The study is conducted in Yavatmal and Chandrapur districts of Vidarbha region of Maharashtra State. Health related surveys were carried out in 54 villages from study area selected on the basis of high fluoride level in groundwater. Result: Out of these, 32 villages are selected from Yavatmal district having a population of 30320 and 22 villages from Chandrapur district with a population of 21581. Out of the total population of 51901 total 3268 subjects have been examined. Amongst these subjects 1760 were male (53.86%) and 1508 were female (46.14%). Out of the total 3268 subjects 2445 subjects included in the ‘normal’ grade, which does not show indications of skeletal fluorosis. It has been observed that as the concentration of fluoride increases the cases of ‘normal’ grade decreases. Out of the 360 subjects studied, about 102 (28.33%) subjects show radiological evidences of skeletal fluorosis. Mild radiological fluorosis was seen in 55, moderate in 21, severe in 17 and very severe radiological fluorosis in 9 subjects. Conclusion: Based on the outcome of the research work carried out in the study area, it is recommended that awareness among the people residing in the area about the protection & prevention of groundwater contamination from different sources, groundwater quality, its impacts on health, soil and plants should be created on priority basis.

Keywords: Fluoride Contaminated Groundwater, Human Health, Fluorosis, Endemic Areas

Background

Groundwater has become the major source of water supply for domestic, industrial and irrigation sectors of many countries. Fluorosis is an endemic health problem in twenty-five nations around the globe (¹,²,³) It is a conclusive fact that concentration of fluoride between 0.6 to 1.0 mg/L is essential in potable water to protect teeth decay and enhance bone development, while their higher concentration may lead to fluorosis (³). Central Ground Water Board (⁴) and Groundwater Survey and Development Agency (⁵) carried out studies on occurrences of fluoride in groundwater in Maharashtra state which falls under second category, where 40 to 70 % of the districts are affected. The endemic districts are Bhandara, Chandrapur, Nanded, Aurangabad and Yavatmal, where up to 13.4 mg/L fluoride has been reported (⁴). Though work pertaining to groundwater quality, hydrogeology, groundwater resource estimation, development and management have been carried out in parts of study area, detailed health related studies including effect of high concentration of fluoride in groundwater on human health have not so far been carried out in the present area. Therefore, objectives of

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the present investigations to correlate fluoride content of groundwater and health status of affected population and suggest remedial measures.

Methodology

In the present study, effect of fluoride on human health is studied and relationship with skeletal and radiological findings is explored. A cross-sectional research design is followed because people from different age groups were sampled, observations were made and skeletal examinations were conducted at a specified point of time.

**Study Setting and Location:** The study is conducted in Yavatmal and Chandrapur districts of Vidarba region of Maharashtra State. The study includes Ghatanji, Kelapur, Maregaon, Wani talukas from Yavatmal District and Warora, Bhadrawati and Chandrapur talukas from Chandrapur district. Prior to the commencement of the study, permission to conduct the research investigations was sought from the Institutional Ethical Committee, Datta Meghe Institute of Medical Sciences (Deemed to be University). Following considerations were taken into account during the planning and execution of the study. The data of fluoride level in groundwater received from Groundwater Survey and Development Agency (GSDA) for study area have been used.

In the present work, BIS recommendations have been used for the desirable level of fluoride in groundwater (1mg/L) as the study area comes under semi-arid climate and surveys have been carried out in rural and tribal part of Yavatmal and Chandrapur districts where most of the population is farm laborers or working laborers in quarries or mines and their per day consumption of water is 3 to 4 liters/day/person. In view of this fluoride level in groundwater has been divided into four groups namely ≤1mg/L, 1.01 - 2.00mg/L, 2.01 - 4.00mg/L, >4.01 mg/L. Safe level of fluoride in the potable drinking water has been considered as 0.5mg/L desirable and 1.0 mg/L as maximum allowable concentration (6).

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Health related surveys were carried out in 54 villages from study area selected on the basis of high fluoride level in groundwater. Out of these 32 villages are selected from Yavatmal district having a population of 30320 and 22 villages from Chandrapur district with a population of 21581. Out of the total population of 51901 total 3268 subjects have been examined. These

54 villages are selected Primary Health Centre (PHC) wise and total 2 villages are selected from 26 PHC, while from Durgapur and Guggus PHC of Chandrapur taluka, one village each is selected. From each taluka two villages are selected having fluoride level ≤1mg/L, 1.01 - 2.00mg/L, 2.01 - 4.00mg/L, >4.01 mg/L. In Chandrapur taluka >4 mg/L fluoride level is not present in the groundwater and therefore two villages from this category have not been selected.

**RECRUITMENT AND SAMPLING PROCEDURE**

A nonprobability convenience sampling technique was used in the study, as the samples were restricted to a part of the population that was readily available and true random sampling would have been difficult to achieve due to time, cost, and transportation limitations. To start with, 3602 potentially eligible subjects were identified which were examined for eligibility. Out of this, 3268 subjects were confirmed eligible and were included in the study. All these 3268 subjects gave consent to participate in the study and so were investigated and their data was analysed. 334 subjects were excluded from the study because they either met exclusion criteria or were not willing to participate in the study because of social reasons or lack of availability of time.

**Data Collection Procedures**

The participants were evaluated according to pre-designed protocol. They were explained the purpose of the study and were requested to participate in it. Each participant was interviewed and examined. The purpose of the questions was to extract a brief medical history and to identify pre-existing risk factors for skeletal deformity. The formulation of the questions was same for all the participants to ensure reliability.

**SKELETAL INVESTIGATIONS**

In the present work, during the clinical examination, base line data was collected using the proforma, while incidence of skeletal fluorosis has been assessed on the basis of physical tests designed for assessing the pain in the joints (2). Classification of skeletal fluorosis based on the clinical and radiological examinations given by Teotia, M. and Singh, K.P. (6) has been used. Out of the
Results and Discussion

In the present work health related surveys were carried out in 54 villages selected on the basis of fluoride level in groundwater. The villages surveyed are 32 from Yavatmal district and 22 from Chandrapur district. Out of the total population of 51901 from these 54 villages, total 3268 subjects have been finally selected and examined. Amongst these subjects 1760 were male (53.86%) and 1508 were female (46.14%).

The fluoride level in groundwater has been divided into four groups namely first ≤1mg/L, second 1.01-2.00mg/L, third 2.01-4.00mg/L and fourth >4.0 mg/L. The participants are also distributed into three age groups. In the first group (≤18years) the subjects included are 1099 (33.63%). In the second age group (19 - 60 years) 1075 (32.89%) subjects and in the third age group (>60 years) total subjects included are 1094 (33.48%) (Table 1.1).

| Table 1.1 Distribution of studied subjects as per age group and F⁻ level in groundwater |
|---------------------------------|----------------|----------------|----------------|----------------|
| F⁻ level in groundwater         | Σ≤1.00 mg/L   | 1.01 - 2.00 mg/L | 2.01 - 4.00 mg/L | > 4.00 mg/L |
| Age Group                      | M  | F  | Total | M  | F  | Total | M  | F  | Total | M  | F  | Total |
| ≤18                            | 13 | 9  | 268   | 129 | 259| 158    | 149 | 110| 259    | 133 | 9  | 291    |
| 19-50                          | 13 | 7  | 256   | 119 | 256| 146    | 123 | 269| 135    | 136 | 151| 271    |
| >60                            | 13 | 5  | 263   | 128 | 263| 145    | 132 | 277| 148    | 119 | 267| 159    |
| Total                          | 411| 376| 787   | 449 | 378| 827    | 432 | 365| 797    | 468 | 389| 857    |
|                                |          |          |       |      |      |        |      |      |        |      |      |        |
|                                |          |          |       |      |      |        |      |      |        |      |      | 100.0  |

Relationship of Skeletal Fluorosis with fluoride Level in Groundwater

Relationship of skeletal fluorosis with fluoride level in groundwater used for drinking purpose has also been studied and their details are given in table 1.2. The concentration of F in drinking water has been divided into 4 groups namely first (≤1 mg/L), second (1.01-2.00 mg/L); third (2.01-4.00 mg/L) and fourth (>4.0 mg/L).

As discussed earlier, out of the total 3268 subjects 2445 subjects included in the ‘normal’ grade, which does not show indications of skeletal fluorosis. It has been observed that as the concentration of fluoride increases the cases of ‘normal’ grade decreases. The remaining 823 subjects show symptoms of skeletal fluorosis. Present study showed that out of the total 823 subjects, 431 show the symptoms of ‘mild’ grade, where 60 subjects are from ≤1mg/L group. This could be due to long time of exposure to F⁻ intoxication and change in source of drinking water. Earlier they were using the drinking water with high concentration of fluoride and now shifted to the drinking water source having low concentration of fluoride.
Study by Teotia, M. and Singh, K.P.\(^{(6)}\) has shown that the clinical recovery of skeletal fluorosis is partially possible with supplementation of vitamin D and calcium. But in the present case as the subjects are from the rural and backward area where the food is habitually deficient in vitamin D and calcium no clinical recovery has been observed. Perusal of table 1.2 shows that as the fluoride concentration in the drinking water source increases, the cases of skeletal fluorosis increases. In the present case in all the grades (mild, moderate, severe and very severe) there is an increase in the number of cases of skeletal fluorosis from ‘mild’ to ‘very severe’ as the fluoride concentration in drinking water source increases. This has also been observed by Nirgude et al\(^{(7)}\) during their studies in Gadag and Bagalkot districts of Karnataka, Punjab, Nalgonda and Rajasthan areas respectively.

Surprisingly, in the areas where fluoride levels of drinking water is not very high and is in the range of 1-2 mg/L, the cases of skeletal fluorosis have been observed. The proposed possible mechanisms for such severe manifestations may be: (a) high atmospheric temperatures (115-116°F) during summer months, (b) hard physical labor activity, (c) poor nutrition, deficient in calories and vitamin C. Similarly, it is now established that diseased kidneys cannot handle fluoride excretion leading to F- toxicity and development of skeletal fluorosis even while consuming low levels of F- in drinking water supplies \(^{(8)}\).

### Radiological Studies

In the present work, classification of skeletal fluorosis based on the radiological examinations given by Teotia, M. and Singh, K.P. \(^{(6)}\) has been used (Table 6.5). On the basis of radiological studies they suggested mild, moderate, severe and very severe grades depending on the basis of intensity of the fluorosis.

Out of the total 3268 subjects studied, 360 subjects are subjected to radiological investigations at Acharya Vinoba Bhave Rural Hospital, Sawangi Meghe, Wardha. Out of the 360 subjects studied, about 102 (28.33\%) subjects show radiological evidences of skeletal fluorosis. Mild radiological fluorosis was seen in 55, moderate in 21, severe in 17 and very severe radiological fluorosis in 9 subjects. In general, in male
the radiological fluorosis is more as compared to female. In ‘moderate’ and ‘very severe’ grades the radiological fluorosis in female is marginally more as compared to the male subjects (Table 1.3).

<table>
<thead>
<tr>
<th>Grade of Fluorosis</th>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>147</td>
<td>71.02</td>
<td>111</td>
<td>72.55</td>
<td>258</td>
<td>71.67</td>
</tr>
<tr>
<td>Mild</td>
<td>34</td>
<td>16.42</td>
<td>21</td>
<td>13.72</td>
<td>55</td>
<td>15.28</td>
</tr>
<tr>
<td>Moderate</td>
<td>11</td>
<td>5.31</td>
<td>10</td>
<td>6.54</td>
<td>21</td>
<td>5.83</td>
</tr>
<tr>
<td>Severe</td>
<td>10</td>
<td>4.83</td>
<td>7</td>
<td>4.58</td>
<td>17</td>
<td>4.72</td>
</tr>
<tr>
<td>Very Severe</td>
<td>5</td>
<td>2.42</td>
<td>4</td>
<td>2.61</td>
<td>9</td>
<td>2.50</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>100</td>
<td>153</td>
<td>100</td>
<td>360</td>
<td>100</td>
</tr>
</tbody>
</table>

**Conclusion**

Based on the outcome of the research work carried out in the study area, it is recommended that awareness among the people residing in the area about the protection & prevention of groundwater contamination from different sources, groundwater quality, its impacts on health, soil and plants should be created on priority basis.

Creating environmental awareness among the people about the habit of safe drinking water, side effects of drinking high fluoride rich groundwater, improving oral hygiene conditions and handling of defluoridation instruments is essential in effective mitigation of fluorosis.

Regular health surveys and Periodic monitoring should be conducted in the affected areas to assess the health impacts of high concentration of fluoride and NO$_3^-$ present in groundwater, especially in infants and children residing in the area.

More emphasis may be given to intake of calcium and phosphorous rich food as it helps in reducing the absorption of fluoride by intestine and also reduces the rate of accumulation of fluoride in human body.

As abundance of fluoride is more in deep aquifers (bore wells) compared to shallow aquifers (dug wells), the groundwater from shallow aquifers are safer and should be preferred for drinking purpose, if nitrate and other contaminants are within the safe limits.

Water quality management in the area should be carried out considering the hydro-geochemical conditions existing in the area and influence of geogenic and anthropogenic factors on physico-chemical characteristics of groundwater.

Defluoridation technique like Nalgonda, reverse osmosis, activated alumina and other techniques may be adopted in areas where no alternative source is available with the involvement of community in operation and maintenance. Recharging centres for the household activated alumina filters for defluoridation of groundwater shall be set up at each affected village.

The planners, policy-makers and public should become more aware of programme concerned with the
sustainable management of groundwater quality.

Integrated fluorosis mitigation approach in the form of dilution of groundwater through artificial recharge, nutritional supplement through food intake and use of cost-effective and environmentally friendly adsorption based defluoridation techniques should be adopted in dealing with fluorosis problems existing in the area.

**Conflict of Interest:** None

**Funding:** Datta Meghe Institute of Medical Sciences

**Ethical Approval:** Obtained from Institutional Ethical Committee of DMIMS, Wardha.

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Quality, Health, and Safety Assurance of Fish Produce based on the Republic of Indonesia’s Governmental Decree No. 57 of 2015 on the Quality, Health, and Safety Assurance of Fish Produce and the Increase of Fish Produce Value: A Sociosphere Analysis in North Maluku

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Abstract

Objective: This paper aims to describe the findings on quality, health, and safety guarantee of fish catch and the related regulations in the Governmental Decree No. 57 of 2015. Method: This research uses the juridical sociologic research method, with the descriptive qualitative approach, which describes the guarantee of quality, health, and safety of fish catch based on the Governmental Decree No. 57 of 2015. Results: It is found that there are violations in the form of an unhygienic method in processing the fish catch. In the Fish Auction Center, the processing uses chemical substances which are prohibited for food. Conclusion: The government should make a policy which support the fishermen, so that they may use a safer and a healthier method in processing fish.

Keywords: Quality Guarantee, Health, Fish, Seafood, Regulations

Background

The handling of the various problems which are the cause of food safety hazard regarding the fish-catching activity started from the activity in catching fish to the end consumers. The food safety system in the fish agroindustry include Good Manufacturing Practices (GMP), Standard Sanitation Operational Procedure (SSOP) and Hazard Analysis and Critical Control Point (HACCP).¹ For the sales of domestic fish commodities or products, the fish catch of the fishermen are directly brought to the distributors to fulfill the demand of fresh seafood or to fulfill the demand of primary or secondary fish-processing industries.

Food quality and safety cannot be separated when discussing about seafood products. This is based on the fact that fish is included as highly perishable food. Thus, there must be high attention to the efforts to maintain the quality and safety of the products. Food products such as fish and its produces must be required to fulfill some food health and safety standards before being consumed.²

The fishermen have a crucial role in maintaining the health and safety standards of those fish. In the food industry, it is a must for the businessowners to pay attention to the safety of the food products so that the products may be accepted with superior quality.³

To guarantee the fish catch quality of health and safety, the Fishery and Sea Service of North Maluku worked together with the Fishery and Health Service of Ternate City to establish the fish catch guarantee of quality monitoring in the fish ports in the Fish Auction Center which was participated by fishermen, processing,
and marketing representatives.

In this activity, the head of the Fishery and Sea Service of North Maluku, M. Buyung Rajiloen, S.H., M.H. (personal communication, June 2020) stated that, “It is crucial to guarantee the health quality of the fish products in this fishery port to increase the fish catch health and safety quality for the people to consume. The increase of fish catch quality by the fishermen, the processors, and the marketers in Madya Ternate city may increase its price, which will surely increase the income of the fishermen.”

The increase of health safety and quality on the fish for consumption may be carried out after the fish caught by the fishermen by using the cold chain system. This system is crucial as fish have a high level of water and protein, which makes them prone to rot. The most effective method is by maintaining the fish’s temperature in a fresh condition when arriving at the port.4

Research Methods

This study used a sociological juridical research method with a qualitative descriptive approach, to describe the quality assurance, health and safety of fish catch based on Government Regulation No. 57 of 2015. Descriptive method is used to describe and explain the factual conditions during the research, examining how the Quality Assurance, Health and Safety of Fishing Products in North Maluku.

Discussion

The government actually has often socialized to fishermen, managers and the marketing department to not cheat in using additional materials which are prohibited by the government such as formaldehyde, rhodamine, and others. This needs to be conveyed in order to maintain the positive aspirations of processed fishery products from North Maluku province, such as Fufu (smoked fish), tore (roasted fish), salt (salted fish), ngafi (anchovies) and balacan (shrimp paste).

Mrs. Ivon as the head of quality development and verification from the Marine and Fisheries Office of North Maluku province conveyed the processing (SKP) from the Health Office of the Madya Ternate city, the head of the Drug and Food Monitoring Ternate Post, Drs. Karim Latu Consina Apt, M. Kes as the head of drug and food control delivered material about the assurance of safety and quality of food quality that will be marketed to the community.

Poor handling practices may lead to microbial contamination and may accelerate the rate of spoilage of fish5. Food safety, especially seafood, continues to be a problem for people around the world. Inappropriate food handling is caused by a lack of knowledge about food safety6. The fish catch requires special handling to keep the fish fresh. Handling of fish on board includes all actions against catches on board, from initial action to storage. This aims to maintain the quality or quality of fish according to the certain standard7.

The fishing method (a type of fishing gear) is directly related to the way the fish die and the way the fish die is related to the physical and chemical processes that the fish body experiences in post-fishing. The government’s concern for quality assurance of fishery products is quite high, it can be seen from the laws and regulations or policies that have been established, namely where these processes have a direct effect on fish quality.8 The poor quality of fish can cause the sales price of the fish to decrease9 as this has been regulated in the Government Regulation of the Republic of Indonesia Number 57 of 2015 concerning the System of Quality Assurance and Safety of Fishery Products and Increasing the Added Value of Fishery Products.10 The cleanliness of the tools, the deck of the ship, the containers used are an effort to reduce contaminants in fish11.

The absence of a clean seawater-cooler machine renders the facility regarded as inadequate or not fulfilling the standard. According to Nurani et al.12, Ships with a refrigerated seawater system or RSW is better in implementing the quality standard compared to ships with a cooling system using ice blocks.

The fish-catching equipment which must be fulfilled by the fish-catching ships are as follows: 1) The tools and equipment must be kept clean; they must be kept in a good condition and must be ready to use. 2) There must be tarpaulin to protect the fish from the heat of the sun. 3) There must be a seawater pump. 4) There must be an adequate supply of ice to fulfill the need in sailing and in handling the fish after being caught. 5) The tools used to handle the caught fish must be maintained well. 6) There must be water/ice used to cool and to refrigerate.13
The requirements which must be met by the fish-catching ships regarding the role of the sailors are as follows: 1) the sailors must have the knowledge and the skills to catch fish, 2) the sailors have the responsibility to maintain personal hygiene and to maintain the ship’s facilities including the tools and equipment, 3) The ship’s crew who handle the fish must be in a healthy condition. s, 4) The ship’s crew must undergo a periodic health examination at least once a year.\(^{13}\)

The handling of the fresh fish by the North Maluku fishermen usually starts as the fish are brought up from their habitat in the sea. They are treated with low temperature. Sometimes, there is a lack of care regarding the factors of hygiene and health, even though the treatment, hygiene, and cooling process are the keys to produce fish catch of good quality. Only a small percent of motor boats bring ice to the sea. The fish hold used for it is usually far from perfect. There is a lack of sanitation and health factors when handling the fish on the boats. They do not keep the fish in a perfect condition, as some do not use chests or bulkheads, which makes the quality of the fish not good as they arrive on land.

Nasran\(^{14}\) states that, “The process of cooling the sea catch is included as a post-harvesting activity. The fishing ships are completed with holds, tanks, and fish containers (chests, drum). But none of those equipment are insulated. They bring some supply of ice and other supporting materials, such as salt, fish-packing materials, etc.”

The technical sampling analysis was carried out at six research locations in North Maluku. It was shown that dangerous additional chemical materials to preserve the fish in the form of formalin was found in three locations. Meanwhile, the rest of the three locations show a negative result. This shows that there are cases where dangerous additional chemicals were found in fresh fish, as concerned by the writer.

As we know, the fish catch products such as shrimp or fish which use the formalin preserving material have some characteristics. The characteristics include having a clean white color, springy texture, the gills are dark red instead of bright red, and the durability increases. Apart from that, the illegal additional chemical material in the form of formalin was also found on dried fish in four locations, meanwhile the other locations show negative results. Meanwhile, there is no proof of the presence of other illegal food additives such as borax and rhodamine B.

As described above, the evidences on those cases have been obtained from the survey results in the visited venues of fish-processing. Usually, the formalin chemical preservative is used on fresh fish or dried fish by the processor to fulfill certain market segments which demand springy and durable fish\(^{15}\).

Technically, the processors consider the effectiveness and the quality of a better preservative, which is usually present in non-food preservatives. For that reason, many of the fish processors use formalin as fresh fish or dried fish preservatives. Apart from that, the use of formalin in food cannot be separated from macro policies which are implemented by the government.

The increase of the gas price which happened also influenced the income of the fishermen. The impact of that policy is seen from the increase of the production price. This makes the solar gas rarer and more expensive. The materials which must be brought to the sea become more expensive, including the ice blocks which are usually used to preserve fish by the fishermen.

Regarding the issue of formalin usage by the fishermen, marketers, or processors in the research venues, most have real impact to the fish demand. The people do not care about the fish they consume. The perception formed is that all fish marketed contain formalin. The impact is that the consumers will be scared or will have antipathy towards fish. Even though not all fish industries will be impacted, but this will make the people concerned as this will have direct impacts towards the income of the fishermen, the processors, or the sellers.

The government needs to formulate policies which support the fishermen, so that they may have the capability to use a healthier and a safer method. Without a pro-fishermen policy, thus the Republic of Indonesia Governmental Decree No. 57 of 2015 on the Quality and Safety Assurance System of Fish Produce and also the Increase of the Fish Produce Value becomes useless.

A one-sided decisive policy which is unbalanced by a pro-fishermen policy will be useless. The fishermen do
not have many choices in maintaining the fish products’ safety and health standard if there is no incentive nor protection in the form of policies which bring advantage to the fishermen.

The sociosphere (the social environment) of the fishermen in North Maluku actually encourage them to use the method which produces an unhealthy and an unsafe sea produce from time to time. They are influenced by a non-populist policy which makes the sailing cost higher and higher. They lost competition to the illegal fishing perpetrators who have better ships and equipment. The healthy method of processing fish becomes less and less affordable.

The sociosphere is the most important environment in determining the health of the environment. The sociosphere is an environment which is formed due to a rational relation between human beings to fulfill the needs or to seek solutions towards the many challenges and difficulties together.

There are some socio-cultural problems which cause the continual malpractice in using illegal chemical products as the additives to the seafood caught in North Maluku. There is a lack of the official authorities. There is a lack of socialization, coaching, and trainings on food safety. There is a low awareness of the processors and the society on the food produce safety and there is a lack of long-term thinking. The society has an eating habit which lacks attention to the safety of the consumed food to their health.

In the institutional aspect, there is a weak coordination, job delegation, authority, implementation, and technical realization in the field regarding the sales and the processing of the food with safety and quality assurance. This is stated by the Minister of Health, Siti Fadilah Supari which suggests that there is a lack of coordination in the National Agency of Drug and Food Control which causes the issue of seafood being preserved with formalin. She suggests that the National Agency of Drug and Food Control have neglected its duty and its authority in monitoring the food and drugs, which may endanger the health of the people.

### Conclusion

The handling of fish on the ship includes all treatments towards the fish catch on the ship, starting from the initial treatment to the storing. This aims to maintain the quality of the fish to match the desired standard. The indicator of a good treatment is that the fish catch has a good quality and is safe to be consumed. A bad quality of fish may cause the low price of the product in the market.

The government needs to formulate more policies which is pro-fishermen, so that they may use a safer and healthier method. The fishermen do not have many choices in maintaining the health and safety standard of the fish and its products if they do not receive incentives and if they do not have protection in the form of policies which put them on the upper hand.

The sociosphere of the fishermen in North Maluku actually encourages the fishermen to use unsafe and unhealthy methods in processing the sea produce. They are influenced by non-populist policies, which inflate the sailing cost. They also lost competition to the illegal fishing perpetrators who own ships and better equipment in treating the sea produce. The healthy processing method of sea produce becomes less and less affordable.

**Source of Funding:** Author

**Ethical Clearance:** Yes

**Conflict of Interest:** No

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Nursing Intervention for Caregivers of Post Autologous Bone Marrow Transplantation Patients at Home

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Abstract

Background: Autologous bone marrow transplantation is a curative procedure for hematological diseases and immune deficiencies. The unique and intensive nature of this treatment requires distinctive care during and post the entire transplant course. Nursing intervention for caregivers post autologous bone marrow transplantation patients at home is an important component of the care that improves and develops knowledge and practice of the caregivers, to take care of the patients in order to help them follow the demanding treatment plan to reach recovery. Aim to evaluate the effect of nursing intervention for caregivers of post-autologous bone marrow transplantation patients at home. Material and Method: A Quasi-experimental study was carried out among convenient sample of 143 patients and their caregivers who attended the follow up clinic during the first six months post autologous bone marrow transplantation. The study was conducted among patients and their caregivers in three different hospitals Sheikh Zayed Specialized Hospital, National oncology institute, and Nasser institute at outpatient’s follow-up clinics. A self-administered questionnaire of patients and their caregivers’ demographic data, and reported checklist of caregivers’ knowledge and practices toward post-autologous bone marrow transplantation patients at home. Results: there was a statistically significant difference between pre and post nursing intervention, which indicate improvement in caregivers’ knowledge and practice post implementation of the nursing intervention program, and positive correlation between their knowledge and practice. Conclusion and Recommendations: Although caregivers had adequate knowledge, practice on some aspects, gaps were identified. There is a need for educational interventions and discharge plan to upgrade knowledge and practices of the caregivers of post autologous bone marrow transplantation patient at home.

Keywords: Autologous bone marrow transplantation, caregivers, nursing intervention, home.

Introduction

Since the onset and progression of bone marrow transplantation it was evident from the 1950s to early 1960s that nurses play an important role within the multi professional team caring for patients and their families go through this treatment.¹ Bone marrow transplant is an umbrella term for the grouping of a variety of procedures. These transplants use stem cells from bone marrow, peripheral blood or cord blood and more recently the procedure has been called haemopoietic stem cell transplantation (HSCT) The type of treatment is then classified based on the origin of the stem cells, autologous being derived from the recipient himself. ² The family does need support, and need pre- and post-HSCT education and psychosocial intervention,. All stages of the transplant process will affect family members. The effect of this severe medical procedure will clearly extend beyond the person concerned to the entire family network. ³ In the post-transplant duration, it is important to have a distinct pattern of evaluation to assess disease condition and for any post-transplant complications.⁴ Increasing caregivers’ confidence and competence require training in the skills they need to provide care to the patient. Past studies have repetitively shown that caregivers often express attentiveness in, and have a need for education and support programs.⁵
The population of Egypt exceeded 100 million in 2020. There are fifteen transplant centers and the transplant rate is 8.4 million. Bone marrow transplantation in Egypt began on a restricted scale in 1989. In 1997, the rate of transplantation increased significantly. Specific attention has developed towards educating the long-term survivors and their caregivers in which nursing intervention plays a significant role because medical treatment activities are more in the background and day-to-day questions have to be dealt with.

Research Hypothesis:

The nursing intervention program will improve the caregivers’ level of knowledge and practices in caring of post Autologous Bone Marrow Transplantation Patients at Home.

Materials and Methods

A quasi-experimental study was carried out among 143 patients and their caregivers at outpatient follow-up clinics in three biggest hospitals in Cairo, Egypt for autologous bone marrow transplantation patients, Sheikh Zayed Specialized Hospital, National Oncology Institute, and Nasser Institute. An official written letter including the title and purpose of the study obtained from the dean of Faculty of Nursing, Ain Shams University to the get the approval form directors of the mention hospitals to conduct the study. A consent was obtained from each participant (patient and caregiver).

The 143 (patients and caregivers) were divided into 6 groups The actual process of data collection was carried out in the period from February 2019 to July 2019 The intervention program consisted of 17 hours (5 hours theoretical, 12 hours practical). Educational media were used such as poster, PowerPoint, laptop, handout Arabic booklet, videos.

The tool was developed by the researcher, based on reviewing related literatures and experts’ opinions, written in Arabic language, and Completed under supervision of the researcher through group interview. A self-administered questionnaire of patients and their caregivers’ demographic data includes 10 questions. Self-Reported checklist of caregivers’ practices and knowledge toward post autologous bone marrow transplantation patients at home. Caregivers’ Knowledge consisted of 15 questions with Cronbach’s value 0.78.

Answers was coded as follow: poor=1, good =2. Caregivers’ practices consisted of 90 questions including:- patient transfer, Central venous catheter care, Meals preparation and diet restrictions, Personal hygiene, Medication administration, and Following Infection control. With Cronbach’s value 0.76.

The total score was divided into two scale: Poor > 60% Good ≥ 60%.

The collected data were organized; tabulated and analyzed using software, the appropriate statistical tests was the Statistical Package for Social Science SPSS (version 25). The statistical analysis includes; percentage (%), Chi-Square test (X^2), Proportion probability P value.

Significance of results was described as follows:

· Not-significant difference obtained at p> 0.05.
· Significant difference obtained at p< 0.0 5.

Evaluation the level of improvement in caregivers’ knowledge, practice, was done by giving post-test similar to pre-test. Evaluation was administered to the caregivers after completion of the program in order to estimate the effect of the program on the caregivers, and recognize the benefit of the program and what are the ways of obstacles to lack of implementation.

Results

Table (1) Shows that, the median and range of age of the studied patients was 48 (21-50) years, in relation to gender, 53.1% were males, and 67.1% were married, while 58% had 3-4 children, as regards educational level 42% of them had university education. Concerning occupation, 37.8% were employees, with 70.6% had sufficient monthly income.

Regarding the caregivers Illustrates that, the median and range of age of the studied caregivers was 47 (18-60) years. 68.5% of the caregivers were females. Regarding Kin-relation with the patient 30.8% were spouse. Related to educational level 41.9 % of the studied sample were secondary education. In relation to the marital status, 41.9% were married, Concerning of job 34.3% were employees, and 76.9% of time of care
were full time.

Figure (1) displays that, pre interventional program good knowledge and practice was 27.2%, 36.4%, respectively, while post implementation of an interventional program good knowledge and practice was 87.5%, 89.3% respectively.

Table (2) Clarifies there was statistical significant relation between pre and post of total caregivers’ demographic characteristics (age, gender, educational level, occupation, time of care) and total caregivers’ knowledge and practice score. However marital status, kin-relation, shows no significant effects on caregivers’ knowledge and practice.

Table (3) Illustrates that there was statistical significant positive correlation between pre & post-test of total caregivers’ knowledge and practices at P < 0.001.

Table (1): Distribution of the patients and their caregivers according to their demographic characteristics (n=143).

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Patients</th>
<th>Caregivers</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Median age, yrs. (range)</td>
<td>48 (21-50)</td>
<td>47 (18-60)</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>76 (53.1)</td>
<td>45 (31.5)</td>
</tr>
<tr>
<td>Female</td>
<td>67 (46.9)</td>
<td>98 (68.5)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>19 (13.3)</td>
<td>28 (19.6)</td>
</tr>
<tr>
<td>Married</td>
<td>96 (67.1)</td>
<td>60 (41.9)</td>
</tr>
<tr>
<td>Divorced</td>
<td>12 (8.4)</td>
<td>29 (20.3)</td>
</tr>
<tr>
<td>Widow</td>
<td>16 (11.2)</td>
<td>26 (18.2)</td>
</tr>
<tr>
<td>Number of children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>21 (14.7)</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>34 (23.8)</td>
<td></td>
</tr>
<tr>
<td>3 – 4</td>
<td>83 (58.0)</td>
<td></td>
</tr>
<tr>
<td>≤ 5</td>
<td>5 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Educational level:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does not Read or write</td>
<td>7 (4.9)</td>
<td>7 (4.9)</td>
</tr>
<tr>
<td>Read &amp; write</td>
<td>3 (2.1)</td>
<td>6 (4.2)</td>
</tr>
<tr>
<td>Primary</td>
<td>19 (13.3)</td>
<td>42 (29.4)</td>
</tr>
<tr>
<td>Secondary</td>
<td>54 (37.7)</td>
<td>60 (41.9)</td>
</tr>
<tr>
<td>University</td>
<td>60 (42.0)</td>
<td>28 (19.6)</td>
</tr>
</tbody>
</table>
Cont... Table (1): Distribution of the patients and their caregivers according to their demographic characteristics (n=143).

<table>
<thead>
<tr>
<th>Occupation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jobless</td>
<td>14 (9.8)</td>
</tr>
<tr>
<td>Employee</td>
<td>87 (60.8)</td>
</tr>
<tr>
<td>Housewife</td>
<td>42 (29.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monthly income</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufficient</td>
<td>101 (70.6)</td>
</tr>
<tr>
<td>Not sufficient</td>
<td>42 (29.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kin-relation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>22 (15.4)</td>
</tr>
<tr>
<td>Spouse</td>
<td>44 (30.8)</td>
</tr>
<tr>
<td>Children</td>
<td>33 (23.0)</td>
</tr>
<tr>
<td>Brother</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Sister</td>
<td>19 (13.3)</td>
</tr>
<tr>
<td>Relatives</td>
<td>9 (6.3)</td>
</tr>
<tr>
<td>Friend</td>
<td>8 (5.6)</td>
</tr>
<tr>
<td>Paid- caregiver</td>
<td>6 (4.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of care</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Full time</td>
<td>110 (76.9)</td>
</tr>
<tr>
<td>Part time</td>
<td>33 (23.1)</td>
</tr>
</tbody>
</table>

Figure (1): Distribution of total caregivers’ knowledge and practice total score level regarding pre and post nursing intervention (n=143).
Table (2): Relation between caregivers’ demographic characteristics and total caregivers’ knowledge and practice score level pre & post interventional program (n=143).

<table>
<thead>
<tr>
<th>Caregivers’ demographic characteristics</th>
<th>Total caregivers’ knowledge</th>
<th>Total caregivers’ practices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td></td>
<td>X2</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>82.500</td>
<td>0.000</td>
</tr>
<tr>
<td>Gender</td>
<td>116.783</td>
<td>0.000</td>
</tr>
<tr>
<td>Marital status</td>
<td>2.228</td>
<td>0.136</td>
</tr>
<tr>
<td>Educational level</td>
<td>124.415</td>
<td>0.000</td>
</tr>
<tr>
<td>occupation</td>
<td>123.779</td>
<td>0.001</td>
</tr>
<tr>
<td>Kin-relation</td>
<td>0.509</td>
<td>0.476</td>
</tr>
<tr>
<td>Time of care</td>
<td>105.187</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Not Significant P >0.05  Significant P <0.05

Table (3): Correlation between caregivers’ knowledge and practices pre & post training program (n=143).

<table>
<thead>
<tr>
<th>Item</th>
<th>Total caregivers’ practice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Total caregivers’ knowledge</td>
<td>0.870</td>
</tr>
</tbody>
</table>
Discussion

The current study illustrated that Table (1) the median and range of age of the studied patients was 48 (21-50) years, in relation to gender, around half of the patients were males, and about two third were married, while more than half had 3-4 children, as regards educational level less than half of them had university education. Concerning occupation, almost one third were employees, with more than two third had sufficient monthly income. This study was in agreement with previous studies. 7,8

Regarding the caregivers Illustrates that, the median and range of age of the studied caregivers was 47 (18-60) years. More than two third of the caregivers were females. Regarding Kin-relation with the patient less than one third were spouse. Related to educational level below than half of the studied sample were secondary education. In relation to the marital status, less than half were married, Concerning of job almost one third were employees, and more than three quarter of time of care were full time. This study was in the consistent with previous studies. 7,9,10 Similar findings also were reported in the studies conducted in USA. Becoming a caregiver was a second full-time occupation, as caregiving had become the priority in their lifetime. 11 But another earlier study was in contrast to the current study, claiming that young caregivers typically have to juggle jobs, their own family commitments, middle-aged caregivers usually worry about missing workdays. Interruptions in work absence leave and reduced productivity. In addition to, limited income can place families at risk of care for non-compliance or of treatment related decision on the basis of income. 12

Concerning caregivers’ demography Table (2) Clarifies there was statistical significant relation between pre and post of total caregivers’ demographic characteristics (age, gender, educational level, occupation, time of care) and total caregivers’ knowledge and practice score level. However marital status, kin-relation, shows no significant effects on caregivers’ knowledge and practice. The finding was supported by other study conducted in USA Indicating that the caregiver does not necessarily discuss the gender position of the participants. This can be explained at least in part by the fact that the majority of caregivers are women, that caregivers themselves are viewed as a woman’s position, and that male caregivers make less use of resources, including support groups, than women do. 5

The same results were observed in early studies that educational and training needs would flow over time, driven by complex processes that characterize adult and family life trajectories. Only a few States provide financial aid to family members who perform the position of caregivers. Therefore, to give full-time care, caregiver has to leave the workforce to support the loved ones. Currently, the Health Care Management System offers some assistance to spouses who perform the function of caregivers. 5,13 With regard to time of treatment. In addition, there were statistically significant differences between the pre-and post-intervention programme, the

Figure (1) displays that, most of the caregivers had good knowledge, and majority of them had good practice post-interventional program, indicating that the caregivers improved in their knowledge and practice after application of nursing interventional program. This could be because they got benefit of the program, and education outcomes. The level of good knowledge and practice were lower in pre-interventional program as mentioned in early study that Family caregivers often feel unprepared, have inadequate knowledge, and receive little guidance from the transplant team. Beside education should pass expertise, skills and details. Successful delivery of education is a dynamic process that depends on sufficient timing. The primary outcome identified in the majority of health education. Respondents show increased health awareness and make healthier decisions about their patients’ health following intervention. So good education also increases patient/ caregiver self-efficacy, reduces anxiety by planning for transplantation, and increase the satisfaction of the patient. More long-term education results might include improvement in survival and transplantation morbidity, accessibility of health care, willingness of patients / caregivers to return to work, and quality of life. 12,13 other study revealed that the preparation of family caregivers for their position must include clear education, including skills training. Preparing caregivers should be an ongoing process such that benefits are maintained and education programmes develop as required. 14
current research in the same line of the previous study showed that full-time caregivers offer more support and can apply the information acquired by the caregiver from the programme.  

There were no major variations in marital status, the relationship of caregivers and their expertise and experience in the current research. The findings show that, in accordance with the previous studies it reported that there was no significant relation between marital status, and kin-relation of caregivers and their knowledge and practice. This may be due to the nature of the intimate relationship between the caregiver and the patient. It did not rely on the relationship. However, depending on the nature of the relationship between the patient and the caregiver, it could possibly lead to a complicated situation of caregiving that could affect the delivery of care, but did not influence awareness.  

Finally Table (3) Illustrates that there was strong positive correlation between pre & post-test of total caregivers’ knowledge and practices, the caregivers’ total knowledge score was positively associated with the total practice score toward post autologous bone marrow transplantation patients at home. The higher the knowledge, the higher the practice, in which this shows that caregivers’ practice is directly related to their knowledge as reported in the previous study. In the most recent sense, a similar study was performed in the United States, which determined that there is a substantial association between the degree of education of caregivers and their practice in post-autologous bone marrow transplantation patients. Improved awareness and/or willingness to offer treatment to United States.  

In other previous study mentioned that Role preparedness has been studied in terms of how the development of knowledge and skills might protect the caregiver from role distress when the difficulty of care or the need for care is high.  

**Conclusion**

Based on the results of the current study and research hypothesis; implementation of nursing intervention program improved the knowledge and practices of the caregivers of post autologous bone marrow transplantation patients at home, there was statistically significant differences between pre/post-nursing intervention post autologous bone marrow transplantation regarding caregivers’ knowledge, practice. In addition, there was statistically significant relations between caregivers’ demographic characteristics and total score level of caregivers’ knowledge and practice in some parts, beside there was positive correlation between pre & post-test of total caregivers’ knowledge and practices score level. Further research with a larger sample of caregivers from other governorates in Egypt are required in order to have a better understanding the needs of caregivers’ knowledge and practices post autologous bone marrow transplantation patient at home.

**Conflict of Interest** – Nil

**Source of Funding**- Nil

**Ethical Clearance** – obtained

**References**


Chronic Kidney Disease: A Case Report

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Abstract

Chronic kidney disease can occur in mostly those patient which having a history of hypertension and diabetes, this disease also known as a chronic renal failure. In this disease condition kidney can decreasing the rate of filteration or kidney function since month or a year. In the beginning period of Chronic Kidney Disease the person not showing any sign and symptoms about disease condition, after the months and year patient showing symptoms like nausea, vomiting, lethargy edema, confusion and loss of appetite. If the early detection and treatment is not getting so the patient can goes in complication such as heart disease, hypertension, bone disease and anemia.1 Case Report: The female patient name is Rashida khan 63 year old religion by Muslim lived in the yawatmal. She was admitted in the AVBRH with the chief complaint of breathing difficulty, fever, nausea and vomiting, swelling on both legs, lethargy weakness since 10 days. She having a history of HTN since 5 year and freshly diagnosed as DM2. Conclusion: This case presented how the prolong history of HTN which is uncontrollable causing the DM2 and it can leads to CKD. The firstly clinical presentation of the patient providing information about the history of HTN and DM2 and the laboratory studies suggested a reducing function of kidney. So the patient sign, symptoms and the laboratory values showing the condition of patient kidney with increasing volume of kidney, breathing difficulty, moderate anemia and uremia. Early detection of the HTN and DM2 and their management in early stage, also the modification in lifestyle and diet can help to reduce the chances of occurring Chronic Kidney Disease. Patient also have to complete the given treatment and counseling about the disease condition, and the problem get solved by taking proper treatment.

Key word: chronic kidney disease, Diabetes Mellitus type 2, Hypertension.

Introduction

Chronic kidney disease can occur in mostly those patient which having a history of hypertension and diabetes, this disease also known as a chronic renal failure. In this disease condition kidney can decreasing the rate of filteration or kidney function since month or a year.1 In the beginning period of Chronic Kidney Disease the person not showing any sign and symptoms about disease condition, after the months and year patient showing symptoms like nausea, vomiting, lethargy edema, confusion and loss of appetite.2 If the early detection and treatment is not getting so the patient can goes in complication such as heart disease, hypertension, bone disease and anemia. If there is not given proper treatment for the Chronic Kidney Disease so it leads to total damage of the kidney.3 As ageing the person get older the chances of kidney diseases is increased and it very common in elder people. The prevalence shows the disease can occure in one men in five men and one women in four women, and mostly occure in age between 65 to 74 years.4 Hypertension, chronic diseases and diabetes mellitus 2 that is the main cause behind the occurrence of Chronic Kidney Disease.

The high prevalence of hypertension in such contries like African and Diabetes Mellitus mostly seen in the South Asian People that is the main reason behind the Chronic Kidney Disease. This condition most commonly seen in the Pakistan, India, Bangladesh and people belong to the black population.5
Treatments may include: Symptomatic treatment like if the person suffering from high blood pressure and DM2 so the providing the antihypertensive medicine and insulin therapy to reducing the complication and deal with Chronic Renal Failure to help in preserving the function of the kidney. Treatment for the chronic renal failure depend on the degree of kidney damage if the kidney in their third degree and more than that so dialysis are the best treatment for preserving the kidney function but when the kidney not showing any response on dialysis so we consider that kidney in very poor condition that time only option that in kidney transplantation. In kidney transplant the receiver get the healthy kidney from the donor into patient body, this kidney can take by living or brain dead donor.

**Case History**

Female patient name is Rashida khan 63 year old religion by Muslim lived in the yawatmal. She is a house wife but she used to do work in her own farm and lived in joined family her husband and son is the breadwinner of the family, rashida done her education in class 8 Th and her monthly family income is around 8000 per month. The source of health care that is government hospital in yawatmal.

She was admitted in the AVBRH with the chief complaint of breathing difficulty, uremia, fever, nausea and vomiting, swelling on both legs, lethargy weakness since 10 days before she came to the hospital she is admitted in yawatmal, she can suddenly unconscious in the house so her relative take her in yawatmal hospital then doctor freshly diagnose as aDM 2 and she also having the past history of HTN. She doesn’t have any other medical history in past and she done her family planning (tubal ligation) other than she not having any type of surgical history.

**Nursing Assessment**

In physical examination the patient showing a poor condition, presence of distress, undernourished, body build thin, activity is dull, conscious and oriented to things. Showing vitals like heart rate of 94 beats per minute, breathing rate of 16 breaths per minute, saturation of 96%, blood pressure that is increase, 140/90 mmHg and she having a fever- 100 F and her body is warm. She having pain in both the lower leg, swelling present on both leg, she had a hemodialysis catheter present over neck. The other physical examination not showing any abnormalities.

Thesome raising and decrease laboratory finding showing below:

- HB% - 10.0 g/dl
- WBC - 15000cu/mm
- Blood Urea - 56.0mgdl
- Bun-26.17 mg/dl
- Creatinine- 5.0 mg/dl
- Sodium – 132 mmo/l
- Potassium – 4.0 mmo/l

**CASE:**

Pantoprazole 40mg- IV-OD
Piperacillin /tazobactam 4.5g - IV - BID
Furosemide - 50mg -IV -TID
Ondansetron - 4g- IV -BD
Insulin - 15mg P/O -BD
Cyanocobalamin - 1amp IV -OD
Nitrofurantoin - 100mg P/O –BD

**Hemodialysis:** she started her heamodialysis cycle as came to hospital,

**Discussion**

Present case report showing that the patient those having the history HTN and it could not control because of not aware about disease condition, adherence of medication, not control over diet and sedentary life style which lead to the DM 2 and this Chronic Kidney Disease of the patient. Then treatment include the drug therapies, control sign and symptoms, Hemodialysis, kidney transplant, patient counseling, prevent complication, life style modification and taking regular follow up. With the help of drug patient need to hemodialysis for recovering the condition of kidney and managing the sigh and symptoms in this disease condition, also reducing the complication which may lead to further. There is need to patient education about her condition and treatment modalities.
All over the world the people present with some degree of kidney disease that is 1 in 10 people. The CKD can develop mostly in elder people but it can develop at any age and there so many condition leads to develop CKD. It can be very common with people increasing their age, nearby 1 % function of the kidney can affect by the age over the 40 year. Such as condition like hypertension, diabetes mellitus type 2, heart diseases can start affecting on the kidney after ageing as compare to young people. In developing countries over the 600 million population they do not afford the surgeries like renal replacement so it can be leads to increase the mortality rate in kidney patients. Although 80 % of people who get the renal replacement therapy can be alive in some developed countries. As we see in our country there is less than 10 % of people we get to need the renal replacement therapy and they live with the get new kidney, the WKD can spreading massage about the importance of the health of kidney.8

Studies shown that there is the one in five men and one in four women that age of 65-75 year, the elder people come in risk factor to getting the kidney injury as compare to younger people. The health of kidney is very necessary because if the kidney is damaged so it can lead to heart attack, stroke and other life threatening condition. In treatment of CKD some cases are required dialysis and renal replacement therapy. The reducing the risk factor and life modification and early detection and treatment that is the way to reducing the mortality and morbidity rate in kidney diseases.

Conclusion

This case presented how the prolong history of HTN which is uncontrollable causing the DM2 and it can leads to CKD. The firstly clinical presentation of the patient providing information about the history of HTN and DM2 and the laboratory studies suggested a reducing function of kidney. So the patient sign, symptoms and the laboratory values showing the condition of patient kidney with increasing volume of kidney, breathing difficulty, moderate anemia and uremia. Early detection of the HTN and DM2 and their management in early stage, also the modification in lifestyle and diet can help to reduce the chances of occurring Chronic Kidney Disease. Patient also have to complete the given treatment and counseling about the disease condition, and the problem get solved by taking proper treatment.

Ethical approval: Not Applicable

Patient Inform Consent: While preparing case report and for publication patients informed consent has been taken.

Conflict of Interest: Nil

Funding: Not applicable

References

Physiotherapy Students Willingness To Report Misconduct To Protect The Patient’s Interests In Chennai

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1Dean, 2BPT IV YEAR Student, 3BPT IV YEAR Student, SRM College of Physiotherapy, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Tamil Nadu, India

Abstract

Background: Applying the ethics in practice is more essential than just learning it. Any sought of misconduct to the patient is not just an ethical issue but it will also degrade the professionalism and it brings a sense of insecurity in patients and this will slow down the progress of treatment. So it is not only important to avoid the ethical issues and misconduct but it is also necessary to stand against ethical issues that happen within the organization that may done by colleagues or higher authorities without any dilemma. Objective: This study aims to find out the difficulties and dilemmas of the physiotherapist in ethical decision making and disclosing the misconduct of their colleagues without any hesitation. Methodology: A close ended questionnaire was used to assess the willingness of the student physiotherapist to expose the misconduct which might be an internal or external disclosure. The questionnaire consisted of two clinical scenarios which were most likely to arise in a work place. Each scenario consisted of 5 questions that rated the severity, likelihood of confronting, internal disclosure, external disclosure to the association and external disclosure to media. The scoring was based on five point likert scale. It was a study that was done with convenient sampling method. The questionnaire was given to 100 Physiotherapy students in Chennai of both the sexes who were willing to participate in the study. Result: From the statistical analysis, there is a significant difference obtained for the likelihood of reporting the manager’s misconduct than colleague’s misconduct. Also their willingness to report internally within the organization is greater than that of reporting externally to the association and to the media. Conclusion: Ethics being a part of Physiotherapy curriculum, these students have well understood the ethical and professional behavior and have good ethical acceptance. Hence we conclude that the students do understand the seriousness of any misconduct of a therapist to the patient and are willing to expose any misconduct they encounter to the internal or external environment.

Key words: Ethics, Students, Physiotherapy, Misconduct, Ethical dilemma

Introduction

Whistle blowing is usually defined as the reporting “by organization members (former or current) of illegal, immoral, or illegitimate practices under the control of their employers to persons or organizations that may be able to effect action” 1. Reporting any sought of unethical behaviour is more inevitable and essential in the field of health sciences as it is concerned with human lives. Whistle blowing largely helps in the reducing the rates of misconduct in future 2,3. But still blowing a whistle against the organisation or colleague or a superior is a major dilemma faced by an individual because when blowing a whistle, it not only involves the whistle blower and the organisation but it also involves the patient who is actually affected, this will lead to deterioration of the hospital’s fame. This will put the whistle blower at risk 1. All though whistle blowing is a moral behaviour, it will risk the life of the one who reports the misconduct. The person who voluntarily reports the misconduct might be exploited and might end up in losing his livelihood 1, 4. Despite these consequences it is to be noted that those who are with high self-ideals are the one to report the misconduct as they are ones intolerable to the unethical behaviour 4. It must also be noted that a person will hesitate to report his friend if found guilty because of their bond or because of the fear that they might get caught one day 5.
When a person is dishonest or unethical during his student life then there are high chances that he will be more deviant during his professional life\(^5\). Any unethical behaviour as a professional will lead to distrust of patients and a sense of insecurity which in turn reduces the progress in the patient condition that will lead to ending up of the professional’s career\(^6\). This is the reason why any sought of unprofessional behaviour has to reported and stopped immediately. Since ethical acceptance begins at the college level\(^7\), it is important to know level of ethical knowledge and their willingness to follow ethics and their ability to make decisions in critical cases and their willingness to report the unethical behaviour that is either conducted by their superior or their colleague. Though this subject is of high importance there are not enough researches done in India to find out about the ethical dilemmas and decision making related to physiotherapy. Our study aims to find out how well the students are able to understand the seriousness of the misconduct of the therapist to the patient and how willing are they to expose any misconduct they encounter to the internal or external environment. Internal disclosure implies reporting misconduct to an higher authority within the organization. External disclosure implies reporting misconduct outside the organization such as law enforcement or media.

### Materials and Methodology

Based on the selection criteria only physiotherapy students of UG final year, PG first and second year and interns those who were pursuing their degree in Chennai were selected as participants. Departmental ethical committee approval was obtained. A total of 100 participants were aimed and a closed ended questionnaire was sent as google forms. Students from colleges without ethics as a part of curriculum were excluded. Initially the participants were asked to fill the demographic data that included the name, age, college they belonged to, and the year they were studying. The questionnaire consisted of 2 clinical scenarios and 5 set of questions were asked under each scenario. The first scenario was related to colleague’s misconduct and the second one was concerned with the misconduct by the higher official. The first question dealt with how far the students were able to understand the seriousness of the situation while the second question dealt with the student’s willingness to confront the misbehaviour to the one who committed it and insisting him to correct what he did. Question 3 and 4 dealt with blowing whistle to the internal environment. Question 5 is related to the blowing of whistle to the external environment. All the questions were rated according to 5-point likert scale. The answer for the first question ranged from “very serious” to “not serious at all”. The answers for the remaining four questions ranged from “strongly agree” to “strongly disagree”.

### Results

The mean differences between the groups were assessed using the paired sample’s test. The data were analyzed using SPSS statistical software, PC version 20.0.

Table 1: Comparison between the respondents’ scores for the two case stories regarding the severity of the misconduct, the likelihood of taking action to change the situation and the indices of internal and external whistle blowing.

<table>
<thead>
<tr>
<th></th>
<th>Case story 1 colleague misconduct (n 100)</th>
<th>Case story 2 director misconduct (n 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAMPLE</td>
<td>MEAN</td>
</tr>
<tr>
<td>1. Severity of the misconduct</td>
<td>100</td>
<td>3.75</td>
</tr>
</tbody>
</table>
Table 1 shows the comparison between the respondents’ scores for the two case stories regarding the severity of the misconduct, the likelihood of taking action to change the situation and the indices of internal and external whistle blowing.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(INTERNAL WHISTLE BLOWING REPORTING TO A SUPERIOR IN THE WORK PLACE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If you decide not to talk to your colleague, or if you have talked to her about the matter and not succeeded in getting her to report the incident, how likely is it that you will go to someone at the center who has the power to intervene, such as the head of physiotherapy ward or ethics comitee,or if snyone is at the rehabilitationcentre</td>
<td>3.51</td>
<td>100</td>
<td>1.010</td>
<td></td>
</tr>
<tr>
<td>If you decide not to talk to the director, or if you have talked to her and not been able to change her mind, how likely is it that you will report the director’s intentions to someone at the center who has the power to intervene, such as the center’s general director or ethics comitee if any one is at the centre</td>
<td>3.78</td>
<td>100</td>
<td>1.069</td>
<td>.010</td>
</tr>
</tbody>
</table>

**TABLE 2: Paired sample test**
Table 1 shows the comparison between the respondents’ scores for the two case stories regarding the severity of the misconduct, the likelihood of taking action to change the situation and the indices of internal and external whistle blowing. From the scores obtained, the likelihood of reporting director’s misconduct is higher than that of colleague’s misconduct.

Table 2 shows the comparison between the scores of the internal and external whistle blowing to the association and to media. From the scores obtained, the likelihood for internal whistle blowing is higher than that of external whistle blowing. There is a significant difference (0.010) between the internal whistle blowing for colleague and director misconduct. There is a significant difference (0.016) between the external whistle blowing for colleague and director misconduct in reporting to the physical therapy association. There is a non-significant difference (0.056) between the external whistle blowing for colleague and director misconduct in reporting to the media.

Discussion

The purpose of this study is to find out how well the students are able to understand the seriousness of the misconduct of the therapist to the patient and how willing are they to expose any misconduct they encounter to the internal or external environment. Totally 100 physiotherapy students with ethics as a part of curriculum were taken into consideration out of which 58 female and 42 male students have participated. From the study it is found that the physiotherapy students witnessed the situations that are harmful to the patients to be very serious. In such situations, they are likely to report to the higher authorities in both cases of misconduct by their colleague as well as manager. However the rate of likelihood to report varied between both the case stories. Hence there was a significant difference obtained by the statistical analysis of the obtained data. From the scores obtained the likelihood of considering the severity of misconduct, confronting the person responsible for the wrongdoing, reporting the misconduct to someone in workplace, to the association...
or to the media for the director’s misconduct was higher than that of colleague’s misconduct. This shows that the students give more importance to the interest of patient rather than supporting the misconduct of colleague or manager. In situations where such misconduct arises their willingness to report seems to be expressed rather than supporting the misconduct. Similarly the scores obtained for internal whistle blowing was higher than that of external whistle blowing. The reason for this might be because the students prioritize to report to the superior authority within the organization than disclosing it outside the organization. Also Abraham Mansbach et al., (2011) has stated that the students prefers and follows a pattern of internal whistle blowing after which the external whistle blowing is being is approached rarely. Anna Myers, (2008) has also stated that it is wiser to whistle blow first internally and then externally outside the organization. It is more ethical and also being loyal to the organization. While also expresses that whistle blowing to an unethical or a deviant act is a core component of an employee and that at all costs whistle blowing to root out a criminal activity which is possibly harmful to the client or the reputation of the institution must be encouraged.

**Conclusion**

Ethics being a part of Physiotherapy curriculum, these students have well understood the ethical and professional behavior and have good ethical acceptance. Hence we conclude that the students do understand the seriousness of any misconduct of a therapist to the patient and are willing to expose any misconduct they encounter to the internal or external environment.

The limitation and recommendation of the study are The answer that was recorded by the participants was just their interest and opinion towards whistle blowing as a student. But the actual response that they give when they are into the situations might vary. In future studies can be done with a comparison between physiotherapy students with and without ethics as a part of curriculum. In future studies can be done with a comparison between physiotherapy students and practitioners.

**Ethical Clearance:** Departmental Ethical clearance was obtained.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**References**


Cognitive Impairment and Its Impact on Quality of Life in Rural Indian Female after Stroke: A Cross Sectional Study Protocol

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Abstract

Introduction: Stroke represented growing social, health care and economic problems. In 2005 vascular cognitive impairment a condition that can be found in 20-30% of stroke patient. Now in 2018-19 over 50 percent of stroke survivors have reported cognitive impairment 6 months after stroke and are associated with poorer quality of life which increase disability. Cognitive functions is identified as a top priority for stroke research. Cognitive impairment affects inadequate ability to focus on the job, recall, understand, prepare, use knowledge, initiate and stop the operation and solve problem. a stroke impaired cognitive function including focus, memory, vocabulary, executive function, perception and orientation of space. Because of abnormality in functional independence and other abnormalities in higher function, cognitive impairment may lead to affect independence. it increases the death ratio, abnormality. Stroke impacts wellbeing dramatically on health system resulting in high costs, and is also considered a global public health problem due to severe disabilities, functional deficiencies and reduced quality of life.

Method: The corrective study is assessing the cognitive impairment and the quality of life. The cognitive impairment will be measured with the use of the MoCA and QoL will be measured by stroke specific quality of life questionnaire. Female stroke patient with age in between 45-65 year who was diagnosed by the physician is included in the study.

Discussion: Stroke is a prevalent condition which affects most of the Indian population. Most studies are done on stroke including male and females both. Many studies have concentrated on cognitive disability after stroke and quality of life in males but no research is available in rural Indian females. The need for the research is therefore to establish the prevalence of cognitive disability in females and their effect on quality of life after stroke.

Key Words: Rural Indian females, Stroke, Cognitive impairment, Quality of life, MoCA , and SS-QoL scale.

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Introduction

Stroke is a leading cause of death and disability in India. It represented growing social, health care and economic problems. In 2005 vascular cognitive impairment a condition that can be found in 20-30% of stroke patient. Now in 2018-19 over 50 percent of stroke survivors have reported cognitive impairment 6 months after stroke and are associated with poorer...
quality of life which increase disability.

The cognitive impairment after stroke is disturbed in any cognitive domain after stroke, executive function, memory, language, visuo-spatial function, visual-construction ability or global cognitive function. Hence, cognitive function is identified as a top priority for stroke research. Cognitive impairment affects inadequate ability to focus on the job, recall, understand, prepare, use knowledge, initiate and stop the operation and solve problem. A stroke impaired cognitive function including focus, memory, vocabulary, executive function, perception and orientation of space. The word cognitive impairment post-stroke is used to describe both mild cognitive impairment and dementia that either occurs 3-6 months after stroke incident. In addition, An approximate 16.9 million stroke cases occurred globally in 2010.

We do not know how many patients with strokes display deterioration or worsening of their cognitive impairment over a period of several years, and we do not have clear prognostic clues to classify those more likely to deteriorate. The estimates modified stroke prevalence range 84-262/100000 in rural areas, and 334-424/100000 in urban areas.

The definition of post-stroke cognitive impairment usually refers to disorders that arise after neuro-radiological examination after symptomatic stroke with associated ischemic finding. The post-stroke depression was found to be cognitive impairment related. It is believed to be linked mortality, reducing functional outcome and quality of life, and may be correlated with multiple factors and psychological mechanisms. Cognitive deficits are a negative prognostic factor that affects behavior and personality. The method such as Montreal Cognitive Assessment is commonly used clinically for examining cognitive disorder. Because of abnormality in functional independence and other abnormalities in higher function, cognitive impairment may lead to affect independence. It increases the death ratio, abnormality.

The physical, social and psychological effects of this disease are severe – approximately 90% of survivors have some kind of impairment. Quality of life in relation to health refers to all types of quality of life affected by diseases. Stroke impacts wellbeing dramatically on health system resulting in high costs, and is also considered a global public health problem due to severe disabilities, functional deficiencies and reduced quality of life.

**Need of The Study/ Rationale**

Stroke is a prevalent condition which affects most of the Indian population. Most studies are done on stroke including male and females both. Many studies have concentrated on cognitive disability after stroke and quality of life in males but no research is available in rural Indian females. The need for the research is therefore to establish the prevalence of cognitive disability in females and their effect on quality of life after stroke.

**Aim and Objective**

**Aim:** To assess the effect of Cognitive Impairment on quality of life in Rural Indian women after stroke.

**Objective:**

3) To find out the Cognitive Impairment in females after Stroke.

4) To find out the impact of cognitive impairment on the Quality Of Life of rural females after stroke.

**Material and Methodology**

Ethical approval will be obtained from the Institutional ethical committee. 40 participants will be selected randomly specially females and assessed for cognitive impairment and quality of life after obtaining consent form.

**Material-**

4) Couch

5) Chair with hand support

6) Immobilizer belt

7) Table

8) Pen

**Method:**

The research project will be conducted in Ravi Nair College of physiotherapy with rural population. It is cross-sectional observational study. All female patients.
who were diagnosed as having stroke and who fulfilled the inclusion and exclusion criteria included in the study.

The corrective study is assessing the cognitive impairment and the quality of life. The cognitive impairment will be measured with the use of the MoCA and QoL will be measured by stroke specific quality of life questionnaire.

Mixed etiologies subject is excluded from the study.

**Instrumentation:**

MoCA, and SS-QoL scale is use as instrument.

The study selected because:

2. It is simple and convenient
3. Does not involve expensive technology or not to costly
4. It is time efficient to perform
5. Easy to understand
6. Reliability of MoCA is 0.75-0.96 , and SS-QoL is 0.65-0.99

**STUDY DESIGN:** Observational study.

**STUDY SETTING:** Ravi Nair College of Physiotherapy, Sawangi(M), Wardha

**PARTICIPANTS:**

**Inclusion and Exclusion Criteria:**

Inclusion criteria:

1. Female stroke patient
2. Age in between 45-65 year who was diagnosed by the physician is included in the study.

Exclusion criteria:

5) The patient having mix – etiologies
6) Elderly female above 67 year
7) Any other psychological disorder is excluded from the study.

**Variables:**

1. MoCA Scale
2. SS-QoL Questionnaire.

**Data Sources/ Measurement:**

Ethical clearance will be obtained from institutional ethical committee (IEC). Participant will be selected by simple random technique. Inclusion and exclusion criteria will be implemented assessment during the hospital stay or within 3 month of stroke. To assess the different aspect of cognitive functions each participant tested approximately 1 and ½ hours session.

MoCA is commonly used method for cognitive evaluation. Eventually the MoCA returns a judgment-based score. The first only requires verbal input and involves orientation, memory and attention assessment. The second portion assesses the naming skills to obey verbal and written orders, writes a sentence randomly and copies a complex polygon. Since the MoCA includes divisions there is no limit of sub-domains. Total total score is 30 and total administration time is around 10-15 min. A cutoff <24 is used to define cognitive impairment.

With the SS-QoL we assessed Life Value

We applied the stroke Specific quality of life scale this method to measure the Quality of Life. The SS-QoL is a particular instrument used to measure health-related quality of life among individuals suffering stroke. It holds 49 objects in 12 domains. Higher values suggest a better quality of life linked to health.

**Data Analysis:**

Data analyzes are carried out using concise and infererse statistics, using unpaired chesquare research students. The program used in the study will be the SPSS 24.0 version, the praphade prism 7.0 version and the degree of significance < 0.005 (p=0.005 m) is considered.

**STUDY SIZE:** 40

**STATISTICAL METHOD:** Simple Random Sampling

**Result**

Upon completion of the study results, statistical
analysis will be estimated.

The result could show the impact on cognitive impairment and quality of life of post stroke patients in rural Indian females. or

The result could not show the impact on cognitive impairment and quality of life of post stroke patients in rural Indian females.

Discussion

Stroke is a prevalent condition which affects most of the Indian population. Most studies are done on stroke including male and females both. Many studies have concentrated on cognitive disability after stroke and quality of life in males but no research is available in rural Indian females. The need for the research is therefore to establish the prevalence of cognitive disability in females and their effect on quality of life after stroke.

Limitations:

It might be difficult to get convince patient for being a part of this study.

Implication:

Outcome of the study shall help the geriatric population to get aware about their condition and health the researcher to plan the treatment protocol.

Generalizability:

study not yet done.

Ethics and Dissemination:

The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review

Source of Funding: There will be no direct support for this research from public and private organization. The department of physiotherapy, at Datta Meghe institute of Medical Science, Deemed to be university will provide material needed for research.

Conflict of Interest: Nil

References


from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6152181/?report=classic
Knowledge, Awareness and Attitude about Breast Lump among Females Attending Mahatma Gandhi Ayurved Hospital

Ishwari Gaikwad¹, Pradnya Dandekar²

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Abstract

Background: Breast lump is considered as carcinogenic unless it is tested and diagnosed as other than this. Today in India, it is one of the main causes of cancer deaths among women. Only early detection and early medical intervention could decrease its mortality rate. Knowledge and awareness of palpation of breast lumps and attitude to get diagnosed at earliest is extremely crucial. Aims & Objectives: Aim is to assess knowledge, awareness and attitude about breast lumps in women attending Hospital OPD. Objectives are to assess the existing knowledge and awareness and to study the attitude about breast lump examination for prevention & early diagnostic measures. Methodology: The proposed cross sectional observational study will be carried out in in Mahatma Gandhi Ayurved Hospital, Salod (H), Wardha. The women visiting OPD will be randomly selected and with their consent will be interviewed personally by a structured and validated questioner. Result: Result will be interpreted on the basis of statistical analysed data. Conclusion: Conclusion will be drawn from the observations and analysis.

Key words: Breast lump, Breast self examination, Clinical Breast examination.

Background and Rationale

In India, the diseases related to palpable mass in breast are the very common. These palpable lumps may include inflammatory conditions, may congenital or may be related to hormonal mastopathy, having traumatic history, benign and malignant neoplastic conditions. Breast lump is the most source of anxiety to a female when it is discovered. Annually, in an average 20,000 of cases of breast diseases are diagnosed.¹ A mass is cancerous until proven otherwise and have to manage by the usual management of a breast mass.

There has been an increase in the cases of breast cancer over the years and it continues to raise steadily². Breast cancer related mortality is seen at an increasing rate. It is the fifth highest due to cancer. It is the most common cause of death among women suffering from cancer.³ In India, interventions need to be done to guide and educate people about the risk factors, importance of screening and management of Breast lumps.

Awareness about the breast cancer and substantial support for the advancement in the diagnosis technology and treatment care for breast cancer is required. Day by day because of available management and early detection strategies the breast cancer survival rate is increased and death rate is going on declining. Benign breast diseases are more seen as compared to malignant and inflammatory⁴. In benign diseases fibro adenoma are more in frequency as found to be almost half of the case in population⁵. Mammography is the diagnostic tool in early stages before the lump can be felt. It is effective technique to diagnose breast cancer in early stages but
not 100 percent in all the time. The reason behind it as there the changes and symptoms might or might not occur. That is why regular screening is so important.

45% of all cancer in females is breast cancer. Breast cancer is very rare in younger group age & it is very aggressive in this category. Survival rate is very low in this category. A survey study at an international level in 23 countries showed that the awareness in younger age is very low as compare to older women. This emphasizes the importance of promoting awareness among young women about not only breast cancer but any palpable mass in breast and strategies available for its screening are very much essential. Early detection plays a pivotal role in the prevention of breast cancer. The techniques which are recommended for precautions to reduce breast cancer and ultimately morbidity and mortality include easily available method as breast self-examination (BSE), Clinical breast-examination (CSE) and mammography. It is need to promote awareness in society not only about breast cancer but any palpable mass find in breast and different techniques available for its screening. Early detection is one good way to prevent breast cancer. WHO has recommended clinical breast examination for the women who are attending the primary health centres for other health problems. There is an urgent need of intervention as to explore the need & way of different ways to detect breast cancer at earliest. So that incidence & mortality can be prevented.

Objective of the study.

1. To assess the level of knowledge and awareness of breast lumps and its early detection strategies in females.

2. To determine the attitude of females towards screening.

Material and Methods

Study design – It will be a Cross sectional observational study. The sample selection will be randomized. A structured and validated questioner will be prepared to assess knowledge and attitude about Breast lumps among women attending OPD of Mahatma Gandhi Hospital will be interviewed personally. Questionnaire administered by an interviewer will be used to survey socio demographic factors, knowledge, attitude and awareness regarding any palpable mass of breast. Before interviewing the verbal consent of the subject will be taken.

Study place: Study conducted in Mahatma Gandhi Ayurved Hospital, Salod (H), Wardha, which is NABH accredited learning Institute under the ambit of Datta Meghe Institute of Medical Sciences University, Wardha, Maharashtra.

Study Population: All the females above age of 14 years attending the hospital were interviewed.

Methodology

The Participants will be explained about the nature and purpose of study. Consent will be obtained from the participants prior to their recruitment in the study. A self-structured questionnaire will be prepared and validated by the subject expert. All the subjects will be personally interviewed on the basis of questionnaire. The study will be conducted from October 2019 to November 2019. In this period, the females above the age of 14 years attending the hospital in the OPD hours 10 am to 1 pm will be interviewed. The non-probability convenient sampling method will be used to collect data. Their data will be collected and analysed statistically.

Inclusion Criteria

Females above the age of 14 years who are willing to give their consent will be selected for study.

Exclusion Criteria

Pregnant and lactating women will be excluded from the study.

Recruitment

Women willing to participate in this study will be provided details regarding the study by verbal explanation. Those who will agree will be provided information and the questionnaire.

Recruited participant must include in eligibility criteria.

Ethical Consideration

The approval of research protocol has been obtained from Institutional Ethical Committee, MGACH & RC, Salod(H), Wardha with letter no. DMIMS (DU)/IEC/
Expected Results

Promoting awareness in women regarding breast lumps and other various health issues will be the main focus. Even though many more programmes are taking care of these issues at government level, this study will focus on the level of knowledge, awareness & attitude about breast lump among women. Result will be interpreted on the basis of statistical analysis.

Discussion

Education that may be formal or informal way plays an important role in this condition. It helps to understand health related issues. The knowledge about importance of Self Breast Examination, Clinical Breast Examination & mammography will helps the society a lot. Discussion will be derived on all these parameters.

Strengths and Limitations - Strength of the study is that this assessment and then planning of intervention is at rural hospital area. This will provide a big advantage to the community.

Conflict of Interest: Nil

Source of Funding: Nil

IEC Ref. No: DMIMS (DU)/IEC/ Sept-2019/8468
Conflict of Interest: None

References

Ethical Issues at the Interface of Physiotherapy Care and Research Practice in Pediatric Oncology—Descriptive Study

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Abstract

Pediatric oncology has a major role in research. This paper discusses about the ethical consequences of physiotherapy care and research in Pediatric Oncology. Empirical ethics is a broad division, grasping, difference in indicating ethics and empirical research. A major Australian tertiary teaching hospital reveals that 65% of patients present with specific indication for physiotherapy, in that only 12.8% are receiving physiotherapy. Indications include in physiotherapy care are pain, lymph-oedema, incontinence, respiratory and musculoskeletal problems also it includes hard to move. Physiotherapy has extensive role in palliative oncology, it also has large preventative, educative and supportive roles to play and provides independent and complementary therapies for physical debility and pain. Pain from reflex spasm and contracture are dealt with Physiotherapists. Founded that no reference based on standard of life and quality-adjusted life years in terms of preventing dependance and avoid hospitalisation. Treatment of pediatric oncology often has physically, socially and psychologically demands. Always there must be some ethical reason for all the decision on treatment and care for a patient with cancer. It is a fact that ethics needs not only all the stakeholders to do the right things but also it needs the one who do it in the right way. Research practice has led us to great advances in pediatric oncology and the proportion is greater in children than adults. Obtaining the informed consent and assent was the most important ethical challenges faced during the research.

Key words: Ethical issues, Pediatric oncology, Empirical research, Physiotherapy care, consent, assent

Introduction

In Pediatric oncology, children were treated for cancer to increase survival rate. Pediatric with oncology overall survival rate has improved to 25% - 75% in last 5-years compare to past 30 years due to improvement in supportive care.1 Pediatric oncology has a major role in research ideas. As a consequences, many Physiotherapist are indulged in both physiotherapy care and research. The national and international protocols, explained the information of treatment procedure for each type of cancer. In research 70% of children present with cancer are enrolled in study they were from developed country and enroll even during their cancer treatment.1 The process of research and Physiotherapy in the Pediatric oncology has more ethical challenges. Aim of this paper to differentiate between the Physiotherapy care and research practice on children with cancer.1 A major Australian teaching hospital reveals that 65% of children presents with particular indication in physiotherapy, in that only 12.8% are getting physiotherapy.2 Indications includes in physiotherapy care are pain, lymph-oedema, incontinence, respiratory and musculoskeletal problems also it includes hard to move.2

Today, the involvement of physiotherapy on Pediatric oncology has specific roles which are evidence based: Prevention - target specific exercise and education programs, Acute and post acute – bio psychosocial to pain management, Institutional and community based rehabilitation - Easy measure (e.g. wheel chair training after spinal cord compression, gait training for neurological dysfunction and Palliative care - Physiotherapy specific skills such as TENS used for relief pain, lymph-oedema and incontinence programs, laser therapy for wounds and ulcer management and maintenance of free movements and physical function.2

The health-care professionals described many ethical issues facing on physiotherapy and research on...
piediatric oncology based systematic literature.

**Methodology**

A study design was descriptive study. Empirical evidence was more popular and most important from a applied ethics, especially in bioethics. It also has broad category grasping different explanation about the content in ethics and empirical research. To gain empirical information, we collected an article based on descriptive study on observation of parents, children and physiotherapist in physiotherapy care and research practice. The experiences about the ethical issue on pediatric oncology research has the goals. The goals are such as research, informed consent, interests and therapeutic misconception. The knowledge of children, parents and physiotherapists in research gives deep understanding in research and the ethical role in research practice. There are different type of empirical research and ethical reflection: firstly - empirical ethics states that the study of people's belief, intuitions, behaviour and information is meaningful in ethics and it has the emerge ethical points; secondly - empirical ethics accept that the methodology (with descriptive methods such as collecting article related experiments, interviews and observation); thirdly - empirical ethics states that the contrast across descriptive and prescriptive study.

**Discussion**

Analysis of 10 studies were reviewed, it has the contrast between research and physiotherapy care in pediatric oncology. It has problem with informed consent and learn about research setting. Its has the consequence of disappear boundary between research and physiotherapy care. Also ethical view has different in treatment and research goals.

Martine C de Vries et al. 2011 described in term of research, the researchers find the information about what would be the best treatment for children with oncology and improve the interest in research in academic merit. Pediatric oncology child therefore undergo the procedure like blood sample, spinal taps and PET scans not related to treatment goals. Ethics has different two-dimensional treatment and research were provide by the various types of ethical principles. Normally parents and physiotherapist will discuss the idea on what the child interest and make it to use concept on consent. And the treatment should given on the interest of the individual child. Respect given for rights incorporates two ethical decision: 1) The individuals were willing to participate in study 2) the person with smaller or less rights entitled to protection. Respect to rights of the individuals they were voluntarily to participate in the research with basic information about the research and the consent. The research and physiotherapy care has the different in methods simultaneously applied to everyday practice of pediatric oncology. It have the consequence for the informed consent and the child with interest are difficulty to identify by the parents and physiotherapists. Pediatric oncology has many difficulty to get an good informed consent process, specially regarding diagnosis and treatment. Informed consent of participation has there own decision making after understanding the treatment protocols and research and make the participant to participate in the both research and physiotherapy care. The consent form has the following: explaining concept and method it has difficult to understand about it. Although the parent has difficulty in understanding about the informed consent in research such as the risk factors, the procedure, the alternate treatment, the duration of study, the right to dropout and the voluntaries to participate. The duty of parents to care the child, the parents are enrolling their children in clinical care and some will take interest to make their child to participate in research. Motivate and support the research to prevent the future generation from the occurrence. The misunderstanding were happen in between research and clinical care of physiotherapy, parents were mix up the research and clinical (treatment protocol) its has the major disadvantage. The study has revealed that 40 - 80% of subjects has misunderstanding of the research. Not only parents are confusion but also the physiotherapist has confusion while conducting both research and clinical session simultaneously.

Liisa Laakso et al. 2006 stated that palliative care provide both positively team based and community based activity. Approaching the advice and care of a therapist has the effectively utilizes and experience the health care and benefit.

Jean-Claude K Dupont et al. 2016 concluded that the wide spread of ethical issues founded in pediatric oncology. It shows the therapists, parents and child take interest to participate in the research. The evidence of
positive and negative effect of participants were offering research in pediatric oncology. Ethical reflection were need to develop in pediatric oncology community among a person with an interest and have the knowledge about it. Empirical ethics has the difficulty to find out the result with individuals studies. Difficulty to document the informed consent because of the misleading communication or misunderstanding about the research. It has the categories such as (misconception, misestimating or misunderstanding).

Cecilia Bartholdson et al 2014 described based on this study finding it has divided into two domains, which has the objectives of study: ethical related and dealing with ethical related concept. Ethical related issues has dealing with truth about the children diagnosis, treatment and prognosis are unknown to children and the parents prevents the truth-telling to the children. Preventing Truth-telling has the ethical issues and stress to the parents. parents and therapists has protect and preserve the truth-telling still the end of the life. The many results of the study has shown the goals achieved by the child were interest to participate in the study. On major, the pediatric with cancer will affect the family, relatives and caregiver. Because the pediatric is present with cancer. The current study has pain relief as the major goals.

lynette Rashleigh et al 1996 stated that the physical dangers occurs in end stage of cancer and that are managed by the trained professional to deal with it, as a part of physiotherapy. In physiotherapy, the main aim is to reduced the pain in cancer patient. To avoid unnecessary and expensive drug intake by cancer people and involve the people in community level physiotherapy by the patients and care givers.

Stacey L et al 2007 concluded that clinical research has been necessary to improve mortality rate and reduce disorder and improve treatment related to the disease in children with cancer. Before that obtaining informed consent and assent from the child participants. It has the major Challenges on pediatric oncology, children and parents to understands about it.

Conclusion

Research will be continued to improve survival rate and decreasing disease morbidity rate in pediatric oncology. Above all studies found difficult in obtaining informed consent and assent from both parents and children to participate. Researcher and therapist found difficulty to make the parents and children understanding about the procedure and the protocol. Major ethical issues founded were parents were confused with treatment protocol in both research and clinical care practice.

Ethical Clearance: Departmental Ethical clearance was obtained.

Conflict of Interest: Nil

Source of Funding: Self

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Age Estimation from Fusion of Manubrium & Xiphoid Process with Sternal Body: A Radiological Study in Living Individuals from Central India

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Abstract

Forensic Age Estimation is an expertise in Forensic Medicine which aims to find out the most accurate way of determining the chronological age of the unknown person involved in judicial or legal proceedings. This study aims to investigate the relationship between the stage of union at Manubrium, Sternum & Xiphoid Process and biological age in Chhattisgarh population. The study was carried out in 270 healthy subjects (135 males and 135 females) aging from 25 to 70 years. The obtained results from the radiographs revealed that the complete fusion of Xiphoid process & Manubrium with the body of sternum is seen in more than & 70% of individuals in the age group of 50-55 years. Females were consistently developing union at a younger age than their male counterparts. Results also suggest that the age of union is found to vary greatly all over the India indicating the need for separate standards of age of epiphyseal union for separate regions.

Key Words: Manubrium, Sternum, Xiphoid, Union, Central India.

Introduction

Age Estimation is necessary in the identification and creation of biological profiles, which can then be compared with suspects, victims or missing persons. Human identification whether living or dead is one of the most crucial tasks in medico-legal practice. The services of Forensic Medicine experts are sought in establishing the identity of the dead, especially in mass disasters. Extreme mutilation, advanced decomposition, skeletal and fragmentary remains, makes the process more complicated. Most of the time, Forensic Medicine specialists have to mainly depend upon bones for establishing the identity. Estimation of age in elderly person is comparatively more difficult than in young persons. The young individual has various factors for age estimation such as physical and morphological features, eruption of teeth, ossification activities and growth of bones which help in establishing the age with utmost accuracy. On the contrary, the elderly persons have very few identifying factors like fusion of sternal bones, fusion of skull sutures, changes that occur in the pubic symphysis, degenerative changes and application of Gustafson’s formula. Age estimation in elderly person has limitations due to paucity of anatomical factors. The present study is an attempt to assess the age of an individual from sternum which is one of the superficial bones, and is spared even in a highly decomposed body. Moreover, it is a bone which can be easily procured from cadavers, without the slightest damage during a routine autopsy procedure.
Aims & Objectives

1. To estimate age from union of manubrium sterni & xiphoid process with sternum.

2. To compare bisexual difference in union of manubrium sterni & xiphoid process with sternum.

3. To compare the findings of union of manubrium sterni & xiphoid process with sternum in Central Indian population with other parts of India on the basis of previous studies.

Material & Methods

The present study was carried out in the Department of Forensic Medicine & Department of Radiology, SSIMS, Bilai, Chhattisgarh (Central India). A total of 270 individuals participated in this study. The subjects included were individuals of 25-70 years of age from Bilai city. They are born to parents living in Central India and have lived here since birth. The subjects do not have any disease/deformity pertaining to bones or chronic disease affecting the general health. Only those cases were considered whose records for date of birth were available. The X-Ray Sternum Lateral View was taken of study cases after obtaining their written informed consent. In this study only bonafide residents, who do not show any disease in respect to anterior chest wall were considered. The female cases were taken less because of poor quality of X–Ray film due to over shadowing of the breast tissue. The status of Fusion of Xiphisternum and Manubrium with the body of sternum was studied. At the end conclusions were drawn, which were compared with available results of various previous studies.

Results

<table>
<thead>
<tr>
<th>Age group</th>
<th>No of Male cases</th>
<th>Male – complete fusion</th>
<th>Male – no fusion</th>
<th>No of Female cases</th>
<th>Female – complete fusion</th>
<th>Female – no fusion</th>
<th>Total % showing fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>30-35</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>15</td>
<td>1 (6.66%)</td>
<td>14</td>
<td>3.33</td>
</tr>
<tr>
<td>35-40</td>
<td>15</td>
<td>1 (6.66%)</td>
<td>14</td>
<td>15</td>
<td>3 (20%)</td>
<td>12</td>
<td>13.33</td>
</tr>
<tr>
<td>40-45</td>
<td>15</td>
<td>3 (20%)</td>
<td>12</td>
<td>15</td>
<td>4 (26.66%)</td>
<td>11</td>
<td>23.33</td>
</tr>
<tr>
<td>45-50</td>
<td>15</td>
<td>5 (33.33%)</td>
<td>10</td>
<td>15</td>
<td>6 (40%)</td>
<td>9</td>
<td>36.66</td>
</tr>
<tr>
<td>50-55</td>
<td>15</td>
<td>12 (80%)</td>
<td>3</td>
<td>15</td>
<td>10 (66.66%)</td>
<td>5</td>
<td>73.33</td>
</tr>
<tr>
<td>55-60</td>
<td>15</td>
<td>14 (93.33%)</td>
<td>1</td>
<td>15</td>
<td>14 (93.33%)</td>
<td>1</td>
<td>93.33</td>
</tr>
<tr>
<td>60-65</td>
<td>15</td>
<td>14 (93.33%)</td>
<td>1</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>96.66</td>
</tr>
<tr>
<td>65-70</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td></td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In males, xiphoid process shows: non fusion in age group of 25-35 years. 80% of individuals show fusion in age group of 50-55 years & 93% individuals show fusion in age group of 55-65 years. Complete fusion is seen in all the subjects in age group of 65-70 years.

In females, xiphoid process shows: non fusion in age group of 25-30 years. 73% of individuals show fusion in age group of 50-55 years & more than 90% of individuals show fusion in age group of 55-65 years. Complete fusion is seen in all the subjects in age group of 65-70 years.

Table 2: Fusion between Manubrium & body of sternum:

<table>
<thead>
<tr>
<th>Age group</th>
<th>No of Male cases</th>
<th>Male – complete fusion</th>
<th>Male – no fusion</th>
<th>No of Female cases</th>
<th>Female – complete fusion</th>
<th>Female – no fusion</th>
<th>Total % showing fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>30-35</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>15</td>
<td>0 (0%)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>35-40</td>
<td>15</td>
<td>1 (6.66%)</td>
<td>14</td>
<td>15</td>
<td>2 (13.33%)</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>40-45</td>
<td>15</td>
<td>3 (20%)</td>
<td>12</td>
<td>15</td>
<td>5 (33.33%)</td>
<td>10</td>
<td>26.66</td>
</tr>
<tr>
<td>45-50</td>
<td>15</td>
<td>5 (33.33%)</td>
<td>10</td>
<td>15</td>
<td>7 (46.66%)</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>50-55</td>
<td>15</td>
<td>12 (80%)</td>
<td>3</td>
<td>15</td>
<td>12 (80%)</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>55-60</td>
<td>15</td>
<td>14 (93.33%)</td>
<td>1</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>96.66</td>
</tr>
<tr>
<td>60-65</td>
<td>15</td>
<td>14 (93.33%)</td>
<td>1</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>96.66</td>
</tr>
<tr>
<td>65-70</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>15</td>
<td>15 (100%)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td></td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In males, manubrium shows: non fusion in age group of 25-35 years. 80% of individuals show fusion in age group of 50-55 years & 93% individuals show fusion in age group of 55-65 years. Complete fusion is seen in all the subjects in age group of 65-70 years.

In females, manubrium process shows: non fusion in age group of 25-35 years. 80% of individuals show fusion in age group of 50-55 years & more than 95% of individuals show fusion in age group of 55-65 years. Complete fusion is seen in all the subjects in age group of 65-70 years.
Discussion

Table 5: Comparison of Age of Fusion with previous studies:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Researcher</th>
<th>Region/ population</th>
<th>Age of fusion (years)</th>
<th>Between Xiphoid &amp; body</th>
<th>Between Manubrium &amp; body</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vasaiya K2</td>
<td>Ahmedabad</td>
<td>50</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Gautam S3</td>
<td>Ahmedabad</td>
<td>50</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Tailor C5</td>
<td>Surat</td>
<td>&gt;40</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Gaur et al7</td>
<td>Pune</td>
<td>&gt;41</td>
<td>&gt;41</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Vora D8</td>
<td>Rajkot</td>
<td>44-45</td>
<td>55-60</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Patel D et al10</td>
<td>Bhavnagar</td>
<td>42-44</td>
<td>59-64</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Manoharan C et al11</td>
<td>Tirunelveli</td>
<td>35-43</td>
<td>Extremely variable</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Reddy R et al12</td>
<td>Bangalore</td>
<td>40-42</td>
<td>55-58</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Present study</td>
<td>Bhilai</td>
<td>50-55</td>
<td>50-55</td>
<td></td>
</tr>
</tbody>
</table>

Summary and Conclusion

1. This study was conducted exclusively on the young indigenous population of Central India (Chhattisgarh).

2. The union of Xiphoid process with sternum in 80% males is seen in the age group of 50-55 years.

3. The union of Xiphoid process with sternum in >70% females is seen in the age group of 50-55 years.

4. The union of manubrium with sternum in 80% males & females is seen in the age group of 50-55 years.

5. Females were consistently developing union at a younger age than their male counterparts.

6. Fusion was delayed by 5-10 years in this study (Central India) as compared to population of South India but is in agreement with some studies done in Gujarat.

7. Central Indian population is of mixed type comprising of various religions and castes. The opinion about age should always be given in the range.

8. For age estimation, relevant joints should be radiologically examined for different centres and opinion should be arrived considering the status of multiple centres.

Ethical Clearance: Taken from institutional ethical committee.

Funding: Article did not receive any specific grant from funding agency

Conflict of interest: Author declares that there is no conflict of interest

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Original Article

Assessment of Knowledge and attitude about Medicolegal Aspect of Organ Donation amongst Medical Students

Pande Varsha1, Nagrale Ninad2, Patond Swapnil3

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Abstract

Organ Donation is one the lifesaving procedure for many critically ill patients with organ failure. Even if the various advanced and modern medical science and technology, there is Scarcity of organs led to big gap between the demand and Supply of organs with number of donors. Present study was conducted At Jawaharlal Nehru medical college DMIMS, Wardha. It was a cross sectional study and second year medical students who were willing to participate included in the study. After taking their informed consent total of 100 students, brief introduction about the scope of our study, general demographic data was collected. Most of the students answered correctly the knowledge based questions on Organ Donation Appropriate knowledge and a positive attitude by health science students could play a crucial role in shaping public perspective.

Keywords: Organ donation, Knowledge, Attitude, Medical students, Donor

Introduction

Health care professionals are the first person to build the a relationship with the family of donor and health care providers have an opportunity to up thrust the option of organ donation. It has been suggested that a positive attitude of health professionals toward organ donation can influence a potential donor family’s decision to consent2

Main reasons for a shortfall in transplantable organs in all countries are Lack of awareness amongst healthcare professionals to identify brain-dead patients as a potential donor is one reason. Organ donation from brain-dead patients is a particularly contentious, following recent legal recognition of brain death within the cultural context of Confucian beliefs3

Nurses, physicians and health care professionals also responsible for requesting organ donation from the family of a brain-dead patient. Since they are in direct contact with patient care and relative’s. Health care professional hypothesized that staff support, knowledge, and training levels would be significantly associated
with organ donation rates.  

The knowledge, skills, and attitudes necessary for physicians and nurses to promote good end-of-life decision-making are widely variable. Active Participation require for those making requests of families for organ donation and should receive training related to procedure.  

**Material and Methods**

This study was conducted At Jawaharlal Nehru medical college DMIMS, Wardha. It was a cross sectional study and second year students who were willing to participate included in the study. After taking their informed consent total of 100 students, brief introduction about the scope of our study, general demographic data was collected.

Complete procedures involved in the study was explained to them and the assurance of confidentiality of the data collected was given. Structured questionnaire on knowledge and attitude was sent by internet to every participant.

**Results:** All 100 students had replied positively, out of these 53% were female and 47% male as depicted in Table 1

**Table 1:** Gender wise distribution of Allied Health Care Professional students

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (n=100)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>47</td>
<td>47%</td>
</tr>
<tr>
<td>Female</td>
<td>53</td>
<td>53%</td>
</tr>
</tbody>
</table>

Most of the students answered correctly the knowledge based questions on Organ Donation related to disease and infective status of patient 98% and 91% respectively. Response about human organ transplantation act and amendments was 75%, 68% respectively.

Response related to consent and matching of Blood group answered correctly by 89% and 94% respectively in Table No.2

**Table 2:** Distribution of Students as per Knowledge of Organ Donation

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Knowledge about of Organ Donation</th>
<th>Response % Answering correctly</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Have you heard of organ donation term?</td>
<td>98%</td>
</tr>
<tr>
<td>2</td>
<td>Organ donation is legal in India</td>
<td>91%</td>
</tr>
<tr>
<td>3</td>
<td>Are you acquainted with the transplantation of human organs act?</td>
<td>75%</td>
</tr>
<tr>
<td>4</td>
<td>Are you aware of the various amendments in transplantation of human organs act?</td>
<td>68%</td>
</tr>
<tr>
<td>5</td>
<td>Organ of a brain dead patient can be donated?</td>
<td>76%</td>
</tr>
<tr>
<td>6</td>
<td>Blood group of Donor and recipient must be matched before donation and transplant</td>
<td>89%</td>
</tr>
<tr>
<td>7</td>
<td>Consent is required In case of Organ Donation</td>
<td>94%</td>
</tr>
<tr>
<td>8</td>
<td>Patients with HIV and HBV can donate organs</td>
<td>96%</td>
</tr>
<tr>
<td>9</td>
<td>Authorized centre is required for organ donation</td>
<td>95%</td>
</tr>
<tr>
<td>10</td>
<td>Organ Donation should be applied to all persons regardless of socioeconomic status</td>
<td>78%</td>
</tr>
</tbody>
</table>
Table 3: Distribution of as per their attitude towards Organ Donation

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Attitude towards Organ Donation</th>
<th>Strongly Agree (%)</th>
<th>Agree (%)</th>
<th>Not sure (%)</th>
<th>Disagree (%)</th>
<th>Strongly Disagree (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Do you feel comfortable to talk about organ donation with family members</td>
<td>88</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Do you give approval to donate organs when you die</td>
<td>68</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Do you give approval to donate your family member’s organs</td>
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<td>4</td>
<td>Do you have doubt that your body will be damaged following organ donation</td>
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<td>5</td>
<td>Do you think it is duty of health care professionals to give information regarding organ Donation to Patient and relatives</td>
<td>82</td>
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Attitude of students about organ donation, almost all respondents agreed on discussing organ donation with family member and most of the participant response positively about donation of organ 88 % and 68 % respectively And also agrees that its duty of health care professionals to give information regarding organ donation to Patient and relatives.

**Discussion**

In modern era, India witnessed its first successful corneal, kidney and cardiac transplant in the year 1960, 1967 and 1994 though the reverberations for organ donation and transplantation. The legislation called the Transplantation of Human Organ Act was passed in India in 1994 to streamline organ donation and transplantation activities. Broadly, the act accepted brain death as a form of death and made the sale of organs a punishable offence.

Pathi et al reveals that there was significant knowledge gap exists amongst the undergraduates and the general population for organ donation. The positive influence of educational intervention emphasizes the need of an intervention to bring positive changes thereby highlighting the significance of health education.

Sucharitha et al found that there is a positive attitude of medical students towards organ donation but there is lack of sufficient knowledge on the topic. Improving their knowledge by including organ donation topic in medical curriculum can help to reduce this gap.

Schaeffner et al found that knowledge and positive attitude in medical education is toward organ donation are more likely to hold an organ donor card and also feel more comfortable in approaching relatives of potential organ donors.

Similarly Marquès-Lespier found in his study that the students of health sciences professions have an
Optimistic attitude towards organ donation. Although a considerable shortfall of knowledge of organ donation amongst is a barrier to their taking the necessary measures to become active donors.

**Conclusion**

A positive attitude and relevant knowledge of health science students could play a crucial part in shaping public outlook. The CBME and Integrated teaching in medical curriculum must highlight and focus on the importance of organ donation, concept of brainstem death. This significantly associated with organ donation rates.

**Ethical Clearance:** Taken from institutional ethical committee.

**Funding:** Article did not receive any specific grant from funding agency

**Conflict of Interest:** There is no conflict of interest

**References**

Prevalence of Associated Factors of Depressive Symptoms among School Going Children’s

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Abstract
Depression is the most often encountered in the world of mental illness. In adulthood these mental disorders do not understand as people do not want to share their mental illness with health care providers, they are fearful of receiving approval from the physician. The community people in the last century did not accept the existence of mental illness to the children. Nowadays some studies can provide evidence of mental illness and treatment, or the medical field is also evolving so that people believe disease and death rates. Earlier studies show that depression is prevalent in adolescents (10% to 60%). The mood disorder (depressive mood) may inform lows all around, people may go to sadness in this condition, and in a severe case, people may end their lives. A person may suffer for a long period in a situation in which he or she feels sad and does not participate in social, personal, professional life. In all life situations, the person feels low and does not feel pleasure in everyday life, so if the individual can feel these things in their life so that they consider depression. Influence of depression mood which is unusual at a particular age. This can lead to negative effects on a friend, social, and family relationship. Most suicide and suicidal behavior can lead to major depressive disorders. Younger depression can lead to chronic waxing and increased risk of major depression in adulthood. Some studies can indicate that depression may occur early in adulthood as opposed to people’s past lives.

Keywords: prevalence, depression, mental illness, suicidal behavior.

Introduction
Depression (major depressive disorder) is a commonly diagnosed health problem that has a negative impact on how you feel, perceive and act. Fortunately this is always treatable. Sadness leads to the feeling of depression and/or lack of enthusiasm for once loved activities. It can contribute to many emotional and physical challenges which can impair an individual’s capacity to act at home and at work Important events in life, such as poverty or job loss, could cause depression. But doctors only see feelings of distress as part of depression if they carry on. Depression is a persistent and not a passing problem. This is made up of episodes where symptoms last for a minimum of 2 weeks. Depression can take several weeks, months, or years to last.

The mood disorder (depressive mood) will explain the emotional lows all around, people with this condition can go through depression, and people in a serious situation can end their lives. In certain cases a person may suffer for an extended period of time because he feels depressed and is not involved in social, family, or professional life. The person who feels low in every situation of life and does not feel joy in everyday life and if the individual can feel these things in their life then...
he / she considers depression. In this particular period the influence of depressed mood is extraordinary. It will have a negative impact on relationships with friends, the social, and family. A major depressive disorder can cause most depression and suicidal actions. The greatest depressive disorder also affects the most suicide and suicidal behaviour. Younger depression can lead to constant waxing and an increased risk of serious adult depression. Some research may indicate that depression can lead to early adulthood as opposed to previous lives.

**Definition**

Depression is a mood condition which implies a lasting sense of depression and lack of interest. This is different from the mood swings that people regularly experience as part of life.

**Prevalence**

Data available show that the point prevalence of depression / affective disorders varies from 1.2% to 21% in clinical-based studies; 3%-68% in school-based studies, and 0.1%-6.94% in community-based studies. India has only performed one incidence analysis estimating the incidence to be 1.6 percent.

**Risk Factors**

Genetics: A family history of depression can increase chances of having a depression. The disease may be passed on, it’s believed. The precise manner in which this occurs is not clear though.

Death or loss: Sadness and sorrow are natural reactions. Nevertheless, these great pressures can often carry with them severe signs of depression, such as thoughts of suicide or feelings of worthlessness.

Conflict: Personal disputes or disagreements between family and friends may lead to depression.

Abuse: It may also be brought about by physical, sexual, or emotional violence.

Events in life: Even good things could make you depressed, like moving or graduating.

Different diseases: Depression often pairs with another disorder, or can be a reaction to it.

Medicines: Depression can be a side effect of medication that patients taking for a particular illness.

Abuse of substances: Up to 30 percent of people who use drugs or alcohol also have depression. Some people misuse drugs when they’re feeling down. In others, heavy alcohol or drug use may cause depressive symptoms.

Other issues: Issues such as social isolation from another illness or family or social group alienation may lead to depression.

**Causes**

Although don’t know exactly what is the causes depression, several factors often correlate with its development. Depression is usually the result of combining recent events with other long-term or personal triggers, instead of an immediate crisis or accident.

Life events: Evidence suggests that chronic long-term unemployment problems, living in an abusive or dysfunctional relationship, long-term alienation or loneliness, constant work-related stress – are more likely to cause depression than recent life stress. However if the person is at risk due to past bad experiences or personal factors, recent events (such as losing person job) or a combination of events may cause depression. Evidence suggests that persistent long-term unemployment issues, living in an abusive or carefree relationship, long-term loneliness or isolation, excessive tension at work – are more likely to trigger depression than recent life stress. However, if the previous negative experiences or personal factors, the patient is still at risk.

**Person factors:**

- The history of a family
- Full personality
- Serious medical condition
- Alcohol and drug use

**Sign and Symptoms**

Depression symptoms typically progress over days or weeks but person can experience an anxiety or moderate depression period that persists for weeks or months in advance. Not everyone who has depression should be complaining about misery or constant low
mood. A person can experience other symptoms of depression, for example, sleep issues. Some would mourn the ambiguous physical signs.

Signs to feel a person include:

$\quad$ A person can experience other symptoms of depression, for example, sleep issues. Some would mourn the ambiguous physical signs.

$\quad$ Lack of interest in daily hobbies and enjoyment. That is a reduced enjoyment performance. This comes with a lack of sexual desire.

$\quad$ It’s a mood irritable. This may be the main change in mood, especially among young people, and among men (For Maori and Pacific minority groups in particular).

$\quad$ Changes in Sleep habits. Sleep deteriorated more often, with difficulty sleeping, sleep disrupted and/or early waking and unable to sleep again. Several men are oversleeping.

$\quad$ Shift in famine. Too many people don’t feel like eating, and may have lost weight as a result. Many people feel hungrier, even after having fun. For those who do more sleep this is also seen.

$\quad$ Less strength, less exhaustion and less exhaustion. Such feelings can be so serious that it seems too difficult to finish even the smallest task.

$\quad$ Physical slowing or restlessness also leads to extreme depression. For periods, the person may sit in one place, and turn, react, and talk very slowly; or maybe they can’t sit still, but hold pace and wring their hands. The same person can experience alternations which are slowing and agitating.

$\quad$ Truth or shame feelings. Thoughts of guilt or worthlessness. People may withdraw from doing things and contact others because they feel bad about themselves.

$\quad$ Hopelessness, and feelings of death. No hope in life can be felt by the individual, the wish that they were dead or feel suicidal.

$\quad$ Talk of danger. People can consider concentrating hard. Person can not read the paper or watch a TV. We can find it very difficult even to make easy daily decisions.

**Anxiety symptoms**

These are very common as a part of depression but these signs usually stop as depression is treated. Signs of fear include:

$\quad$ Excessive restlessness or anxiety associated with physical signs such as stress in the body, dry mouth and heart attack.

$\quad$ Attacks over panic. Sudden bouts of intense panic and anxiety, including signs of physical cravings.

$\quad$ Falun Gong. Concerns unique to circumstances, items or creatures

$\quad$ The physical wellbeing issues are severe.

Symptoms include depressed mood, diminished participation in play activities, attention issues, anger and behavioral problems such as aggression, pessimism, decreased appetite, reduced sleep, anhedonia, and somatic symptoms should include. 8

**Treatment**

Depression can’t go anywhere, by itself. However, if left untreated and ignored, depression can continue for months, even years, and can have many negative effects on a person’s life.

Each one has to find the care they need. Finding a workable cure will take time and patience.

Various forms of depression demand different treatment. Mild symptoms can be soothed by:

---->Knowing the Condition

$>Changes$ $in$ $lifestyle$ $(for$ $example$ $regular$ $physical$ $exercise)$

$>Psychiatric$ $care$ $offered$ $by$ $a$ $mental$ $health$ $professional$ $or$ $by$ $online$ $e$-therapy.

Medical treatments, in combination with these other treatments, are likely to be needed for moderate to more severe depression.

Depression treatment should start with a patient’s condition. Book an extended consultation to give time
to discuss symptoms and options for treatment. The doctor may ask to complete a screening questionnaire or perform certain tests to rule out other conditions.

Patients should be referred to a psychologist, social worker, counselor or psychiatrist by a doctor. Access a rebate through Medicare to see most of those professionals. This requires that physicians write a patient GP Mental Health Plan.

- Local community health center-get in touch with someone at your local council for details
- Local triage mental health service – they can give you advice about your nearest major hospital with a psychiatric department with mental health assessment staff available.

**Psychological treatments for depression**

Psychological therapy (also called speech therapy) have been shown to be an successful way to combat depression. We will help people adjust thought habits and develop their coping skills, so that you are better able to cope with life’s pressures and conflicts.

Through understanding and modifying unhelpful thoughts and behaviors, psychological therapy will help you remain well.

Several types of psychiatric therapies exist including:

- Cognitive behavior therapy ( CBT)
- Interpersonal (IPT) therapy;
- Comportemental therapy
- Cognitive therapy (MBCT) focused on alertness.

CBT is among therapies that are most widely used. This helps people with depression monitor and change negative thought patterns, and increases their ability to cope better with life’s stresses and conflicts.

A large variety of appropriate treatments are available for the major depressive disorder. Relevant medications (see Medication) and brief psychotherapy (e.g. cognitive-behavioral therapy, interpersonal counseling) can be used to relieve depressive symptoms. Scientific evidence also accounts for the ability of brief psychotherapy (CBT) to prevent relapse.

However, pharmacotherapy by itself is ineffective care in children and adolescents. Nevertheless, the combination of medication and psychotherapy typically provides the quickest and most effective solution in all patient groups.  

**Complementary therapies**

The word alternative therapy is widely used to describe methods and therapies that vary from conventional Western medicine, and can be used to support and maintain it. Some natural therapies will improve your life, and help patients stay healthy. It has been shown in general that attentiveness, hypnotherapy, meditation, exercise, relaxation, massage, mirimiri and aromatherapy all have some effect in alleviating mental distress.

**Complications**

Depression can get worse if left untreated and can lead to problems affecting any part of your life such as social, mental, behavioral, and physical.

- Panic assaults
- Social anxiety or a phobia that results in social isolation
- Weight loss or excessive weight gain
- Physical disorders and illnesses
- Attempts and suicidal thoughts
- Substance misuses like alcohol and medications
- Can affect the immune system

**Prevention**

Even if evidence-based care could be given to all individuals afflicted by a depressive illness due to the constant influx of new patients and the limited efficacy of medications currently available, the effect on averting disabled years will be marginal.

Prevention will offer new ways of reducing the burden of depressive disorders on the disease. Research by the Institute of Medicine described prevention as an initiative aimed at preventing new cases of mental
disorders that occur in individuals that are not yet meeting the criteria for such disorders. 13

**Conclusion**

Depression is the commonest condition in primary care, but it mostly undiagnosed, unrecognized and undiagnosed because of that prevalence will be an increase in the mental disorders. Depression having a high rate of mortality and morbidity when it was untreated. Most of the patient those who are suffering from depression never accept the condition and they feel afraid to get treated, but when the condition goes worse that time it will showing the sigh and symptoms that under controlled by the person. Psychotherapy that is the first line and effective treatment for the depression in mild and moderate phase but when a severe case is their so give treatment along with psychotherapy and medications. Those patients are live with depression along with their family and friends.

**Ethical Clearance:** Taken from IEC-ref.no.DMIMS (DU)/IEC/Dec-2019/8645

**Source of Funding:** Self

**Conflict of Interest:** Nil

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9. WebMD Medical Reference Reviewed by Smitha Bhandari, MD on September 05, 2019


Bibliometric Analysis of Publications on Dental Caries and Dental Fluorosis from India

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Abstract

Introduction- Dentistry goes back to about 5000 BC when a “tooth worm” was believed to be the source of dental caries. Repeated processes of acid generation lead to the superficial degradation of calcified tissue in the tooth, and consequently cavitation. Work has shown that enamel demineralisation happens at a pH of 5.5 and below. World Health Organization (WHO) mission is Health for all. Fluoride containing drinking water and supplements such as tablets, gums, gel, and toothpastes are the major sources that could be responsible for increased fluoride consumption. At various periods of tooth growth, the ingestion of unnecessary fluoride may have a number of consequences on teeth, including the presence of white lines or streaks on enamel, yellow or brown streaks on enamel, and the general participation of enamel with white or dark chalky stains.

Material and Method- A systematic Search Strategy was framed using the specific keywords related to Dental Caries and Dental Fluorosis. The Web of Science Database was accessed and Search Query was input as “KP= (“Dental Caries*” OR “dental fluorosis*” OR “school children 8-13 Years*” OR DMFT* OR “Dean’s fluorosis Index*”)

Result- Search output generated total 210 documents from 111 Sources (Journals, Books, etc.) Over the period from 1999 – 2020. Total Author’s Keywords (DE) were 664

Conclusion- Dental caries is genuinely a neurological disease whose nature primarily depends on the existence of fermentable sugar, host factors, cariogenic microbial flora and other related environmental conditions. Researchers have proposed numerous theories within the context of dental caries. Fluoride is among the relatively few contaminants that may cause significant public safety impacts in drinking water. Various types of exposure to fluoride have been found to influence systemic F material, thereby raising the likelihood of fluoride sensitive diseases.

Keyword- Dental Caries, Dental fluorosis, Publications

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Introduction

Dentistry goes back to about 5000 BC when a “tooth worm” was believed to be the source of dental caries. Around 1634 the expression “dental caries” first appears in the literature, which came from the Latin word “caries,” which stands for decay. Originally, the word was used to identify the gaps between the teeth. Dental caries have been reported to be among the earliest and
most common diseases that exist in humans. Dental caries is a common, chronic, transmissible disease that develops from specific bacteria that attach to the tooth, namely Streptococcus mutans that metabolize sugars to produce acid that demineralizes the tooth’s surface.

Dental caries is genuinely a neurological disease whose nature primarily depends on the existence of fermentable sugar, host factors, cariogenic microbial flora and other related environmental conditions. Researchers have proposed numerous theories within the context of dental caries. Another such hypothesis, suggested by W.D. in 1881. Miller is “chemo-parasitic theory,” which explains the cumulative impact of bacteria that create acids and acids in the oral cavity, and is universally accepted with modification. Using this hypothesis as the basis, many models have been suggested to address the theoretical aetiology of dental caries, such as J.L Williams, Keyes and Fitzgerald’s idea of plaque-causing dental caries to clarify the likely causal association of the involvement of different microorganisms such as lactobacilli, streptococci in dental plaque, and incidence of dental caries. A basic Venn diagram which consists of three circles and the motion of these circles will clarify the aetiology of dental caries. Two circles reflect fruit, plaque, or microbial charge, and in the sixth, the host takes position. Those three circles are intersected by caries. Recently a fourth “time” circle was added which determines the duration of the above circles’ interaction. Plaque and nutritional influences interdepend with one another in the development of dental caries. Through contrast, the third circle, which represents the host, acts as a mechanism for transmitting these variables.¹ The initiation and progression of dental caries involve specific microorganisms. Streptococcus mutations (S. mutans) have main connections with the initiation of dental caries, and Lactobacilli has connections with the development of dental caries. The polymers for these microbes are fermentable sugars, as well as the reserve of biofilm carbohydrates generated by bacteria. When the bacteria metabolize these substrates they create the lactic acids and other acids. In accordance with host factors, lactic acid synthesis reduces the oxygen coefficient locally, which facilitates the occurrence and growth of dental caries. Repeated processes of acid generation lead to the superficial degradation of calcified tissue in the tooth, and consequently cavitation. Work has shown that enamel demineralisation happens at a pH of 5.5 and below. World Health Organization (WHO) mission is Health for all. Universal Declaration (1998), recognizes that all members of the human family have ‘equal and inalienable rights.’² Dental caries is one of the common and most widespread illnesses of humans. While continuous efforts have been made to reduce its prevalence, it is still prevalent, especially in the lower socioeconomic groups. Globally, dental caries is a widespread problem of the disease. The prevalence of dental caries ranges from 49% to 83%, according to a recent survey by the Global Oral Health Data Bank. Regardless of age, almost all age groups are negatively impacted by dental caries. Data from different studies found that teenagers between the ages of 12 and 19 had the largest amount of dental caries accompanied by babies, and then adults. Kids are vulnerable to “rampant caries” or “nursing bottle caries” that mainly involve one or more decayed teeth in every primary dent between the birth date and 71 months of age.³ ⁴ While dental caries is in decreasing patterns in many sections of the developed world, in most developing countries it is still a major public health concern, affecting 60-90 % of school children claim a World Health Organization (WHO) study. In a transition from norm to a more westernized lifestyle, cultural, social and political developments in the developed world have had a major effect on food and wellness. Instead, services are shown to be insufficient for some key prevention initiatives. In fact, lack of knowledge and enthusiasm in the area of public health appears to be significant contributors to the rise in dental caries.⁵ India ranks among the world’s 25 nations, with ever-increasing increases in dental caries. There, dental caries continues as a charred disease that really has spread its tentacles widely into many regions where there have been insufficient dental care facilities, lack of public health awareness, motivation and increased usage of processed carbs.⁶ Dental caries is also a smoldering disease in developing countries such as India, which has extended its tentacles deep into regions where resources are inadequate for dental treatment, lack of medical awareness and incentive for increased consumption of carbohydrates.⁷ ⁸ Low wages, weak oral hygiene, schooling for mothers, fluorosis, enamel deficiency, multiple low socioeconomic interventions, low level of parental education and cariogenic diet all influence caries risk.⁹ ¹⁰ Approximately 60-65 % of the present...
incidence of dental caries in India is rising day by day. Geographical area plays an important role in caries prevalence; this fluctuates with changing location. Caries incidence in India was 51.9 percent, 53.8 percent, and 63.1 percent, respectively, at 5, 12, and 15 years, according to the 2004 National Oral Health Survey. Present, in various regions of India. A varied image shows the frequency of dental caries in India including its accessible literature from 1940 to 1960. Notwithstanding contradictory estimates, the incidence of dental caries was reported to be 55.5 per cent in India in 1940, and 68.4 per cent in 1960. Dental caries and dental fluorosis are double problems for school children in India, “fluoride is sometimes referred to as a double-edged weapon” – the effective and judicious usage of which provides optimum safety for caries, while unfair and unnecessary systemic intake will contribute to lingering fluoride toxicity. That manifests as dental fluorosis and skeletal fluorosis. In the literature, dental fluorosis was defined as a “developmental disruption caused by sustained exposure to high levels of fluoride during teeth development, resulting in the formation of enamel with reduced mineral content and increased porosity.” Fluoride (F-) is among the relatively few contaminants that may cause significant public safety impacts in drinking water. Various types of exposure to fluoride have been found to influence systemic F material, thereby raising the likelihood of fluoride sensitive diseases. Consumption of Fluoride has beneficial effects on small doses of teeth (< 1.0 mg / L), such as avoiding or reducing the likelihood of dental caries, which is one of the main worries among dentists. Such ion may deter new caries from developing, and could even cause some minor cavities to be retrieved. Increased ingestion of fluoride during enamel forming helps in enamel mottling this mottle enamel is also known as dental fluorosis. It causes visible differences in enamel (intrinsic tooth discoloration), which often contributes to actual injury to the teeth. The effect of the condition will vary according to the dosage, duration range and age of the individual during exposure. The esthetic portion of mild to extreme fluorosis is of significance as it allows the teeth to experience physical harm. Fluoride affects the dental caries forming cycle by three ways: enhancing the chemical enamel structure throughout its production and rendering it more immune to acid attack; promoting mineralization with increased enamel crystal quality; and the plaque microorganisms’ capacity to generate acid. However, too much fluoride absorbed for longer times (higher than 1.5–2 mg / l) may induce dental fluorosis. Fluoride containing drinking water and supplements such as tablets, gums, gel, and toothpastes are the major sources that could be responsible for increased fluoride consumption. At various periods of tooth growth, the ingestion of unnecessary fluoride may have a number of consequences on teeth, including the presence of white lines or streaks on enamel, yellow or brown streaks on enamel, and the general participation of enamel with white or dark chalky stains. Children below the age of 13 have oral health as the object of several epidemiological studies carried out around the world. According to the World Health Organization (WHO, 2013), the importance attributed to this age group is that it is the time that children leave primary school.

Methodology

A systematic Search Strategy was framed using the specific keywords related to Dental Caries and Dental Fluorosis. The Web of Science Database was accessed and Search Query was input as “KP=(“Dental Caries*” OR “dental fluorosis*” OR “school children 8-13 Years*” OR DMFT* OR “Dean’s fluorosis Index*”)”. The results were refined by Countries/Regions for India. A list of total 210 articles was retrieved which was imported R-Studio application and analyzed for various categories as outlined in the results.

Results

Search output generated total 210 documents from 111 Sources (Journals, Books, etc.) over the period from 1999 – 2020. Total Author’s Keywords (DE) were 664. Main data revealed following details-
The types of published documents obtained from Web of Science Database were as follows:

- Journal ARTICLE - 161
- ARTICLE, BOOK CHAPTER - 5
- ARTICLE, EARLY ACCESS - 3
- EDITORIAL MATERIAL - 2
- PROCEEDINGS PAPER - 1
- REVIEW - 37
- REVIEW, BOOK CHAPTER - 1

The trend of Annual Scientific Production was as below:

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The Annual Percentage Growth Rate of publications was 11.03.

Corresponding Author’s Countries included India with highest (187) documents followed by United Kingdom (6), Saudi Arabia (5), USA (3), Malaysia (2) and Australia, France, Germany, Nigeria, Portugal with 1 author each.

Fig.1: Country Collaboration Map

Fig.2: Conceptual Structure Map
Fig. 3: The 3 Field Plot

Fig. 4: Keyword Co-occurrence
Discussion

The published materials of Health Sciences University show an increase in the year 2019 whereas the sum of total citations of published materials show a prominent rise in the year 2018. The sum of total citations of materials published in 2019 is expected to show sharp rise in the year 2020. Among all the publish mater Indian authors shows highest number of publication on the topic.

Conclusion

Dental caries is genuinely a neurological disease whose nature primarily depends on the existence of fermentable sugar, host factors, cariogenic microbial flora and other related environmental conditions. Researchers have proposed numerous theories within the context of dental caries. Fluoride is among the relatively few contaminants that may cause significant public safety impacts in drinking water. Various types of exposure to fluoride have been found to influence systemic F material, thereby raising the likelihood of fluoride sensitive diseases. Consumption of Fluoride has beneficial effects on small doses of teeth (< 1.0 mg / L), such as avoiding or reducing the likelihood of dental caries, which is one of the main worries among dentists.

Conflict of Interest - None

Source of Funding – None

Ethical Clearance - Taken from Institutional Ethical committee Datta Meghe Institute of Medical College.

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Effect of Finger and Hand Exercises among Amateur Pianist

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Abstract

BACKGROUND: Now days the number of students studying piano in tertiary institution is being increasing. The piano player is similar to athletic activity to play the notes accurately. Posture plays a vital role in piano. Musicians are prone to injuries due to the nature of musical practice, inappropriate body postures and potentially harmful playing techniques. Majority of amateur players are prone to injuries .Amateur piano player experience pain in hands and fingers. The main objective of the study was to strengthen the finger and hand muscles to reduce the level of pain.

Methodology: Quasi Experimental study and convenient sampling. 20 amateur piano players, were selected according to inclusion and exclusion criteria. Pre test was done to the participants using V AS score, hand grip dynamometer and cornell musculoskeletal discomfort questionnaire. Finger and hand Exercise were given to the participants for 4 weeks . After 4 weeks of study to analyze their level of pain, a hand held cornell musculoskeletal discomfort questionnaire, V AS score and hand grip dynamometer were used to find the effect of finger and hand exercise exercise.

Outcome Measure: CORNELL MUSCULOSKELETAL DISCOMFORT QUESTIONNIARE, V AS SCORE,HAND GRIP DYNAMOMETER

Result & Conclusion: The study concludes that there was significant reduction in the level of pain and also the hand grip was significantly improved

Keywords: Amateur Piano player, harmful playing techniques, finger and hand pain,

Introduction

Piano is a classical and fascinating instrument. In 18th century Bartolomeo Christofori invented first generation of piano and twentieth century piano was highly developed and its quality , both in mechanism and sound reached its peak .Playing Piano notes accurately is similar to the athletic activity. The touch of each key weights around 52g for present day acoustic piano. Piano needs an application of greater muscle effort to stabilize the finger joints¹.

Most of the public music is often perceived as fun and safe job without posing any harm to the body . There is a possibility for musicians to suffer from injuries. Pianist are prone injuries due to nature of musical practice.

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The occurrence of “Playing related musculoskeletal disorders” is almost unavoidable when a given motion is highly repetitive when combined with the prolonged use of body segments and without a proper understanding of the anatomical limits and motion ranges of the Human body.

From the history of piano playing, some experience regarding injuries was recorded such as Robert and Clara Schumann and Sergei Rachmaninov. In twentieth century when piano was highly developed and its quality both in mechanism and sound reached at its peak, many contemporary pianists reported their symptoms and their horrible experience after injuries were developed such as Glenn Gould, and Leon Fleisher (Mark, 1999). In most of cases, injuries can be so severe that they cannot even perform and play some kind of repertoire³.

The present research has shown that Prevalence of PRMD has been reported in the range of 25%to 93%.

Now days students learning music in tertiary institution
has been increasing. The students require extreme mental and physical endurance strength to perform. Although piano techniques vary in different genres of music, examples of faulty piano techniques that could put pianists at risk of PRMD include fingers pressing too hard on the keyboard, head deviating from the central position, elevated shoulder, curving of the wrist which should be in a straight line with the fifth finger, forearm raised until it is not parallel with the floor, elbow too close or too far from the body, and locked wrist.

A regular practice session on daily basis is required for a pianist to reach the required standard. Some piano pieces require more grip and strength in fingers, for example pieces like “MODERATO” AND ALLEGRO. So to play the notes accurately the students practice a lot. A sudden increase of practice time before examination and performance is one such cause of PRMD. Lack of finger exercise prior to and after piano playing is also a risk factor of PRMD. As well, a lack of warm-up and cool-down exercises prior to and after piano playing is also a risk factor for PRMD. The main objective of the study is to strengthen the finger and hand muscles and to reduce the level of pain.

Materials and Methods

The study is an Quazi Experimental study which was conducted in 2020 at Kingsway Music Academy. In this study twenty amateur piano students of both men and women between the age 18 -21 were recruited. The Grade exam appearing students who practice for 60 mins a day and who had Playing related musculoskeletal disorder in Hand and wrist were recruited. The students who learn other instrument along with piano were excluded. Initially the level of pain and hand grip was measure using Vas Score and Hand grip Dynamometer and Cornell musculoskeletal discomfort questionnaire was given to disclose the discomfort in the hand among the participants.

The exercise protocol had been charted out, for 4 weeks duration, twice a day, weekly thrice, 3sets,10 times repetition. Stretching exercise for forearm and finger flexors, lumbricals and thumb extensors with 5 seconds hold. Free exercises such as finger opening and closing, finger taps and thumb bending exercises were charted for the participants. Elastic band resistance was used to strengthen the finger and thumb abductors. Digi flex finger strenghtener was used for strengthening MCP and interphalangeal joint flexor.

Then after 4 weeks of exercise, the Vas score was measured to analyze the level of pain and Hand grip dynamometer to measure the hand grip. Cornell musculoskeletal discomfort questionnaire was given to disclose the discomfort in the hand among the participants.

Result

A total 20 participants were selected in which 11 were women and 9 were men. Out of 20 students 25% were 18 years of age ,25 % were 19 years of age ,25% of them 20 years of age and 25% of them were 21 years of age.

From Table III the pre and post test value, analyzed according to Cornell musculoskeletal discomfort questionnaire. In Area A the mean value of pre test was 5.05 and post test was 2.750. In Area B mean value of pre test was 9.05 and post test was 4.925. In Area C mean value of pre test value was 2.55 and post test value was 2.125. In Area D the mean value of pre test value was 0.50 and post test value was 0.250. In Area E the mean value of pre test value was 3.20 and post test value was 1.8255. In Area F the mean value of pre test value was 4.50 and post test value was 2.050. The pre and post test values analyzed using questionnaire show that pain has been significantly decreased.

From Table IV the level of pain and hand grip was analyzed. The mean value of vas score in pre test was 5.15 and post test was 3.750 in which the level of pain had been significantly decreased. The mean value of hand grip in pre test was 10.25 and post test was 12.500 in which hand grip has been increased.

Table I: Age Frequency

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
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<tbody>
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<td>5</td>
<td>25.0</td>
</tr>
<tr>
<td>19</td>
<td>5</td>
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<tr>
<td>20</td>
<td>5</td>
<td>25.0</td>
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<tr>
<td>21</td>
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<tr>
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<td>20</td>
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</table>
TABLE II: GENDER FREQUENCY

<table>
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<th>PERCENTAGE</th>
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</thead>
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<tr>
<td>FEMALE</td>
<td>11</td>
<td>55.0</td>
</tr>
<tr>
<td>MALE</td>
<td>9</td>
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TABLE III: CORNELL MUSCULOSKELETAL DISCOMFORT QUESTIONNAIRE (PRE & POST TEST)

<table>
<thead>
<tr>
<th>Area</th>
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<th>N</th>
<th>Std. Deviation</th>
<th>t – Value</th>
<th>P - Value</th>
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<tbody>
<tr>
<td>A</td>
<td>5.05</td>
<td>20</td>
<td>4.186</td>
<td>3.467</td>
<td>0.003 S</td>
</tr>
<tr>
<td></td>
<td>2.75</td>
<td>20</td>
<td>3.0414</td>
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<tr>
<td>B</td>
<td>9.05</td>
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<td>2.350</td>
<td>5.133</td>
<td>0.000 S</td>
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<tr>
<td></td>
<td>4.925</td>
<td>20</td>
<td>3.3611</td>
<td></td>
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</tr>
<tr>
<td>C</td>
<td>2.55</td>
<td>20</td>
<td>3.720</td>
<td>0.838</td>
<td>0.412 NS</td>
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<tr>
<td></td>
<td>2.125</td>
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<td>3.2842</td>
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<tr>
<td>D</td>
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<td>1.000</td>
<td>0.330 NS</td>
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<td>4.895</td>
<td>1.729</td>
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<td>20</td>
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<td>F</td>
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<td>4.560</td>
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<td>20</td>
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TABLE IV : VAS AND HAND GRIP (PRE & POST TEST)

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<th>Std. Deviation</th>
<th>t – Value</th>
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<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.15</td>
<td>20</td>
<td>.671</td>
<td>6.294</td>
<td>0.000 S</td>
</tr>
<tr>
<td>Post</td>
<td>3.750</td>
<td>20</td>
<td>.9665</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAND GRIP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10.25</td>
<td>20</td>
<td>2.552</td>
<td>-3.943</td>
<td>0.001 S</td>
</tr>
<tr>
<td>Post</td>
<td>12.500</td>
<td>20</td>
<td>3.8044</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Piano, the hammer incorporated in it strikes the strings and reproduces the sound of musical note, when the key pressed stronger, the velocity of the hammer striking the strings is increased and more sound will be produced(1) this 20th century many young population particularly school going students are interested to learn playing piano as an recreational activity and as an passion on music.
The musculoskeletal injuries in work and recreational activities like sports are common, similarly playing musical instruments is also prone to cause musculoskeletal injuries called Playing-Related Musculoskeletal Disorders (PRMDs).

This PRMDs is common among amateur piano players, mainly due to the posture which assumed and maintained during playing the piano, the wrist and hand symptoms are common among them. There are three types of wrist posture commonly assumed during playing piano, one is high wrist, neutral wrist and weight piano playing. The first one high wrist is preferred by few piano players which is traditional, in this wrist will be higher than the keyboard, the second one is neutral wrist in this the piano player assumes the wrist in neutral posture, the wrist corresponds appropriately to the level of keyboard, the third one weight on piano in this wrist rotates while playing and weight of the hand and wrist will be on the fingers. While playing piano along with wrist and fingers, the elbow and shoulder joints is also involved. While playing piano the posture assumed by the fingers can be flat or rounded, posture of elbow can be straight or bent and posture of shoulder can be elevated or non-elevated. The motion of the fingers can horizontal or vertical while playing piano. Apart from the joints of upper extremities, there will be involvement of motion of neck and torso while playing piano(2).

The wrist joint connects the hand an sensory organ to upper extremity. Numerous muscles, tendons, nerves and vascular structures of hand transverses wrist joint. The neutral wrist joint posture plays a crucial role for normal functioning of these structures, which transversing it and in preventing injuries to these structures from mechanical origin.

The commonly assumed wrist postures during playing the piano are high wrist, neutral wrist and weight of piano. These postures are not an neutral posture for wrist joint, the neutral posture of wrist is forearm in mid prone position, wrist joint neutral with out any flexion, extension, radial and ulnar deviation(5).

In all the three postures which commonly assumed during playing piano, the forearm will be in a fully pronated position, in which radius shaft will be twisted on ulna and with arthro kinematic changes in the inferior radio-ulnar joint such as radius will be migrates medially and superiorly on ulna and ulna abducts and migrates posteriorly(4).

In high wrist posture and weight on piano playing the wrist will be in flexion, the proximal carpal row of wrist will moves volarly and distal row palmarly(4).

In all the three postures of wrist while playing piano, in the MCP joints of fingers, the proximal phalanx will slides and rolls palmarly on flexion and volarly on extension, the proximal phalanx and slides and rolls laterally during abduction, in the corresponding metacarpals. In the interphalangeal joints of fingers, the phalanx will be rolling and sliding palmarly and volarly on finger flexion and extension movements. The first metacarpal of thumb slides and rolls on trapezium palmarly on abduction, medially on flexion, laterally on extension.(3)(6)

The repetitive motion and sustained posture of wrist while playing piano and the arthro-kinematic changes due to it, in inferior radio-ulna joint, carpal joints, MCP joints and interphalangeal joints interfere with structures transversing the joint, commonly there will be length-tension changes in the musculo-tendinous structures and it is prone to cause muscle fatigue, myofascial trigger points, tendinitis and etc. Most commonly the long flexors and extensors of wrist and fingers, thenar muscles, lumbricals, palmar and dorsal introssei muscles are involved. At times the neural structures adjacent to these musculo-skeletal structures is also involved in causing pain and numbness.

The wrist posture other than the neutral will over tax the musculo-tendinous structures and it is prone to cause PRMDs and loss of grip strength(7). Same time the repetitive motion of the fingers and thumb are prone to PRMDs. The long flexors and extensors of wrist and fingers are affected by non-neutral wrist posture and repetitive motions, the intrinsic musculature are affected by sustained finger flexion and abduction and repetitive motions.

The muscular structures of wrist and hand are primarily involved in causing PRMDs among piano players. The PRMDs is more prevalent among adult and children amateur piano players. The common cause of the PRMDs among the amateur players are improper posture of wrist assumed during practice and playing.
lack of strength and flexibility in wrist and finger muscles.

In this study 20 amateur piano player, both male and female genders, between the age group of 18 to 21 years, who had PRMDs in wrist and hand due to playing piano had been selected in and around chennai. In this study on pre-test The pain had been measured with VAS scale, the hand grip strength were measured with hand held dynamometer and cornell musculoskeletal discomfort questionnaire was given to disclose the discomfort in the hand among the participants.

The exercise protocol had been charted out for this 20 participants, for 4 weeks duration, twice a day, weekly thrice, 3 sets, 10 times repetition. Stretching exercise for forearm and finger flexors, lumbricals and thumb extensors with 5 seconds hold. Free exercises such as finger opening and closing, finger taps and thumb bending. Exercise with elastic resistance band to strengthen finger and thumb abductors and finger play strengthener for strengthening MCP and interhalangeal joint flexors. After 4 weeks charted exercise protocol, there was significant improvement among this 20 piano players pain and discomfort in wrist and hand.

**Conclusion**

The study concludes that lack of warm up and cool down exercise among amateur pianist is the cause of PRMD. The study also concludes that Correct positioning at the piano can play a large role in achieving correct balance and posture creating tension free playing. The possible prevention of future occurrence of playing related musculoskeletal disorder is to seek a set of suitable playing technique that is user friendly. Along with posture warm up and cool down exercise can be charted for the amateur piano players so that level finger pain, and fatigue can be minimized.

**Limitation**

Sample size is less.

Limited age group was included.

Protocol Duration was lesser.

**Recommendation**

Interference with large sample.

Exercise Duration could be increased.

Motion capture analysis camera can be used

Appropriate Questionnaire can be designed

**Conflict of Interest:** Nil

**Source of Funding:** Self Funding

**Ethical Clearance:** Institutional Ethical Committe

**References**


17. Sara C. White Prevalence and Risk Factors Associated with Musculoskeletal Discomfort in Spay and Neuter Veterinarians 2013 3(1) 85-108;.
19. Senthil Purushothaman 2019 Relationship between the hand discomfort with the dimensions of hand and touch screen mobiles 2019 12 (3) 537-540.
Analysis of Posture Using Posture Screening Mobile Application among Collegiates

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Abstract

BACKGROUND: A posture is a position of the body which is maintained by the balanced muscular forces on the skeletal system. The posture is affected by the imbalance between the muscle force and also by reduction or increase in the muscle length. OBJECTIVE: To analyze the posture using posture screening mobile application among collegiates. METHODOLOGY: It is a non-experimental, observational study, sample size 200 subjects both genders between 19 to 23 years, more than 18.5 to 25 ratios of BMI were conveniently included in the study. The exclusion criteria was any congenital or acquired musculoskeletal problems and recent surgeries fractures and injuries. METHOD: A written informed consent was taken from the subjects. The posture assessment was taken using posture screening mobile application and was documented. RESULTS: The result shows the percentage of abnormal posture deviation among normal BMI collegiates. CONCLUSION: The study concluded that the prevention of musculoskeletal disorders among young individuals with normal BMI is one of the challenging issues among health care professionals.

Keywords: posture, musculoskeletal disorder, posture screening mobile application

Introduction

Musculoskeletal disorders describe the conditions that affect the muscle bones and joints. Many studies and literatures quotes that the prevalence of musculoskeletal disorder among students are increasing. The factors that contribute to musculoskeletal disorder are imbalance in biomechanics of body, improper posture, weak muscles and endurance¹.

A posture is defined as position of the body or the arrangements of the body segments relative to one another². The posture can be either static or dynamic.

During static posture, in certain positions such as standing, sitting, lying and kneeling, the body and its segments are balanced and maintained. Dynamic posture refers to the posture in which the body or its segments are capable of arranging and rearranging body segments to shape a wide variety of postures, but keeping an upright bipedal position which is ideal for humans, including movements such as walking, running, punching, jumping and lifting. Every specific posture research includes kinetic and kinematic analysis of all body segments³.

Classical method of assessing the posture, in order to understand the optimal posture, it is necessary to understand the optimal alignment of the spine and other joints at rest. This is usually done by analyzing or using plumb line, the gravity should pass through specific points of the body⁴.

On examination, the body should be viewed from three aspects anterior, posterior, lateral. The standard erect posture is where the gravity of the vertical line drawn through the center of gravity of the body is viewed from each side. The gravity line passes through the inner auditory meatus through the shoulder joint and approximately halfway via the cervical and lumbar vertebral bodies. At hip, it passes through the hip joint at the level of greater trochanter of femur. Then it

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continuously passes through anterior to the knee joint and anterior to the lateral malleolus.

When it is viewed from either the front or back, the vertical line passing through the center of gravity should theoretically bisects the body into two halves, the body weights distributed evenly between the legs.

Erect spine must be kept in a position by active forces, counteracting the pull of gravity and the other forces, acting on it. The abdominal muscles remain quiescent although the lower fibers of the internal obliques are active in order to protect inguinal canal.

To maintain an ideal posture, one should possess a good stability and balance with minimal stress. Posture such as lordosis, kyphosis, swayback, straight back, flat back and increased kyphosis with forward head, flat upper back and neck posture, scoliosis are some examples of faulty postures. A forward head posture or poking chin are more common among collegiate students and this may result in craniofacial pain, headache, neck ache and shoulder pain together with decreased range of cervical motion, muscles stiffness and tenderness.

The importance of adapting to an ideal posture in the young age prevents the occurrence of musculoskeletal disorder among collegiate students. A study done has proved 48.6% and 66.8% of students has prevalence of upper cross syndrome and lower cross syndrome. This is an alarming sign which may affect the student’s performance and quality of life. The method of assessing or evaluating the posture among the students are concerned with limited quality. The classical method could help identifying the postural abnormalities but exact degree of deviations are not known.

By assessing the exact degree of deviations, it could help us to work on educating the ideal posture. Each individual may vary with their postural deviation. Thus, applying the standard protocol results in identifying these deviations, it could provide a better outcome.

The technological improvement over a decade has made it possible. The POSTURE SCREENING MOBILE application tool in the mobile is used because of its simplicity, low cost and non-invasive technique applicable in both clinical and community interventions. There are many studies related to postural changes in obese students but there are few studies reported on college students with normal Body Mass Index. Thus, this study will be more useful to assess the posture by using posture screening application. To analyze the posture using the posture screening app among collegiates.

**Methodology**

The ethical clearance for the study was obtained from the institutional ethical committee. It is an non experimental study which was conducted in the year 2019 at SRM Institute Of Science And Technology. In which 200 healthy subjects of both gender, between 19-23 years of age were who were not affected by any physical and mental disorder and who came under the normal bmi range of 18.5 to 25 were recruited for the study. The participants were explained about the procedure and informed consent was obtained. The Posture Assessment was carried out using an Android mobile phone with an Android application “Posture Screening Mobile Application. This posture mobile application has shown high reliability and validity. For postural assessment and photographic documentation, the subjects were requested to stand and the posture was analyzed by using the anatomical landmarks as given in the application. Head posture, shoulder posture, hip posture, and knee posture was assess. A photographic record was taken in both anterior and lateral view. The data collected from the students were tabulated and entered in MS-Excel spread sheet. The data was analyzed using descriptive statistics.

**Aim and Objectives**

To analyze the posture using the posture screening app among collegiates.

During the early adult hood usually, students are neglecting their posture and they are not much aware of its consequences which leads to the muscular imbalance and pain.

There are many studies related to postural changes in obese students but there are few studies reported on college students with normal Body Mass Index. Thus, this study will be more useful to assess the posture by using posture screening application.
Results

A total of 200 participants were selected in which 61.5% were men and 38.5% were women, about 30% of total population were 23 years of age, 20% each at the age of 22 & 21 years and 15% each at the age of 20 & 19 years.

From the anterior view aspect out of 200 participants, In Head 31% had normal deviation and 69% of them had abnormal deviation. In shoulder 12.5% had normal deviation and 87.5% had abnormal deviation. In Hip 25% had normal deviation and 75% had abnormal deviation.

From the lateral view aspect, In Head 12.5% had normal deviation and 87.5% of them had abnormal deviation. In shoulder 75% had normal deviation and 92.5% had abnormal deviation. In Hip 16% had normal deviation and 84% had abnormal deviation. In knee 3% had normal deviation and 97% had abnormal deviation.

### TABLE I: GENDER DISTRIBUTION OF 200 SUBJECTS

<table>
<thead>
<tr>
<th>GENDER</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
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<tbody>
<tr>
<td>NO. OF MEMBER</td>
<td>61.5%</td>
<td>38.5%</td>
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### TABLE II: PERCENTAGE OF PARTICIPANTS BASED ON THEIR AGE GROUPS AMONG COLLEGIATES

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<thead>
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<th>AGE RANGE</th>
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<tr>
<td>20</td>
<td>15%</td>
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<tr>
<td>21</td>
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<td>22</td>
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</tr>
<tr>
<td>23</td>
<td>30%</td>
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### TABLE III: PERCENTAGE OF ABNORMAL POSTURE IN ANTERIOR VIEW AMONG COLLEGIATES

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<tbody>
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<td>HEAD</td>
<td>69%</td>
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<tr>
<td>SHOULDER</td>
<td>87.5%</td>
</tr>
<tr>
<td>HIP</td>
<td>75%</td>
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TABLE IV: PERCENTAGE OF ABNORMAL POSTURE IN LATERAL VIEW AMONG COLLEGIATES

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<tr>
<td>SHOULDER</td>
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</tr>
<tr>
<td>HIP</td>
<td>84%</td>
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<td>KNEE</td>
<td>97%</td>
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TABLE V: PERCENTAGE OF NORMAL AND DEVIATED POSTURE AMONG COLLEGIATES

<table>
<thead>
<tr>
<th>Area</th>
<th>View</th>
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<th>Abnormal</th>
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<td>HEAD</td>
<td>Anterior</td>
<td>31%</td>
<td>69%</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>12.5%</td>
<td>87.5%</td>
</tr>
<tr>
<td>EAR LOBE</td>
<td>Anterior</td>
<td>12.5%</td>
<td>87.5%</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>7.5%</td>
<td>92.5%</td>
</tr>
<tr>
<td>SHOULDER</td>
<td>Anterior</td>
<td>25%</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>Lateral</td>
<td>16%</td>
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<tr>
<td>HIP</td>
<td>Lateral</td>
<td>3%</td>
<td>97%</td>
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</table>

Discussion

The current study explores the postural deviation of the collegiate students with the mean age of 23. To identify the risks of developing musculoskeletal disorders using POSTURE SCREENING MOBILE APPLICATION.

Generally postural deviation is related to the spine and also involves its malalignment. If the body parts are out of the alignment for longer duration, this results in the two ways that one muscle group get shortened and other lengthen in position, this affects efficiency of muscular system and results in musculoskeletal problems.

Many efforts have been taken to educate people about the postural abnormalities and the effects of poor posture, that still remains a major problem among students populations14.

The current findings demonstrate, about 69% of subjects have abnormal posture of head in anterior view 87.5% have abnormal posture in lateral, 87.5% of subjects have abnormal posture of shoulder in anterior view and 92.5% have abnormal in lateral view. 25% of subjects have abnormal posture of hip in anterior view and 75% of subjects have abnormal in lateral view. 97% of the subjects have abnormal posture of knee in lateral view among 200 collegiate students with normal BMI.

On analyzing the posture previous studies reported certain factors in posture modifications among collegiates due to overuse of smart phone gadgets, high heels, sitting or standing, with heavy back packs for a long duration, adopting posture while watching televisions, working in
computers, motorcycles accidents.

Carrying bags in asymmetric positions causes shifts in upper back and shoulder, increase in the cervical lordosis and these factors also contribute to pain and abnormal posture in students\textsuperscript{15}.

The incidence for development of musculoskeletal discomforts are higher in knee region, followed by shoulder region with rounded shoulder. The participants are also at the risk of developing forward head posture.

To compensate these discomforts the students further adapt some abnormal postures which may lead to serious problems.

The poor postural habits are the results in the era of technology which has become a part of the current lifestyle. Engaging the peoples on more of physical activities, exercises and sports could only be alternative ways of maintaining an ideal position.

The methodology of assessing the posture using POSTURE SCREENING MOBILE application really worked well to identify the problems of each individuals.

**Conclusion**

This study find out the prevalence of musculoskeletal disorder among collegiates with normal Body Mass Index. The study concludes that there is increases in risk of postural changes in targeted population. Hence the musculoskeletal pain in young people should not be ignored, as it can help to evaluate pain in adulthood. This study results hold higher risk of postural changes in normal BMI collegiates.

Thus, assessing posture using mobile application paves way to simple, cost-effective and evidence based diagnostic tool. Application of such tool helps health care professionals to design individual intervention protocols.

**Limitations**

- Study is based only on normal Body Mass Index.
- Equal number of gender populations are not taken.

**Recommendations:**

- Utilization of the mobile application in the clinical practice.
- Applicable for research with larger samples.
- Posture screening mobile application can be used as prognostic tool in the field of physiotherapy interventions.
- Awareness among young students about increasing musculoskeletal disorder is more essential.
- Further study are suggested with large number of equal gender populations of the same age is recommended.

Knowledge of health education about occurrence of musculoskeletal disorder among adulthood is important.

**Conflict of Interest:** Authors Have No Conflict Interest

**Source of Funding:** Self Funding

**Ethical Clearance:** Institutional Ethical Committee

**References**


Cognizance Among Physiotherapy Clinicians and Students in Pelvic Girdle Dysfunction- Qualitative Analysis

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Abstract

Low back pain of pelvic girdle dysfunction origin is most prevalent among all age group, hence there is constant need of updates in diagnosis and therapeutic approaches, manual physiotherapy gained an adequate importance in addressing pelvic girdle dysfunctions, with an objective of understanding the awareness of pelvic girdle dysfunctions among the clinicians and students, a self-administered questionnaire was designed and administered in pre-conference workshop pelvic girdle dysfunction dogmas in diagnosis and approach at SRM college of physiotherapy SRM Institute of Science and Technology Chennai India, based on the response and through the statistical analysis it was found that the awareness was lacking and there was also need to focus on the clinicians and student community towards pelvic girdle dysfunctions and its basics, this may be beneficial in enhancing better patient care services and evidence in framing up clinical practice.

Keywords: Manual Physiotherapy; Pelvic biomechanical models; Pelvic girdle dysfunction; Physiotherapy curriculum; Structural diagnosis; Workshop survey

Introduction

The pain and motions in the pelvic girdle structures, had been controversial and still being debatable, particularly from sacroiliac joint and its different types of motion, after several researches and world congress on low back and pelvic pain(1,2), the healthcare community accepted, that sacroiliac joint is prone to cause pain and the joints of pelvic girdle structures poses minimal motions. Researches till date had more emphasized on stability and mobility of these joints, when coming to mobility of sacroiliac joint it is still debatable and the enigma still persists.

The pelvic girdle structures are composed of three bones namely two innominates and one sacrum, the sacrum is invertedly pyramidal in shape base on above and apex on the bottom, superiorly articulate with L5 and inferiorly articulate with coccyx through sacro-coccygeal joint, sacrum has paired articular surfaces for articulating with ilium called auricular surface it is in L-shape with long and short arms, the articulation of sacrum with the ilium through sacro-iliac joint(1). The innominate bone is embryologically divided into three distinct parts ilium, ischium and pubis, ilial part of innominate will articulate with sacrum through ilio-sacral joint(1), the pubis bone articulates with another pubis bone through symphyseal pubic joint, fibrocartilage disc is interposed between the symphyseal pubic joint(21). The three bones and it’s three articulation are clinically significant.

The pelvic girdle structures plays vital role in transferring weight of upper half of the body to the lower extremities and it is an house of abdomino-pelvic visceral contents. The pelvic girdle structures are transversed by various muscles, fascia’s, ligaments and neurovascular structures(3). The dysfunctions of pelvic girdle structure are prone to cause locomotory dysfunctions as well as uro-gynaecological problems(1,3,4).
physiotherapy discipline which predominantly deals with the human pathokinesiological problems has an pivotal role in addressing the Pelvic Girdle Dysfunctions (PGD). The physiotherapy specialities in Orthopaedics, Obstetrics & Gynaecology, Sports and Biomechanics has wider scope in evaluating and addressing the pelvic girdle dysfunctions.

In India only few physiotherapy clinicians are evaluating and addressing PGD with manual therapy approach appropriately. Most of the physiotherapy clinicians either misdiagnose this dysfunction or address PGD symptomatically with conventional electrotherapy modalities. Ignoring these structures dysfunctions or addressing with electrotherapeutic modalities will not give long term results. Consequences of unaddressed malaligned structures can lead to orthopaedical problems such as wear and tear of hip, pes-anserinustendinitis, foot problems and etc (1,3,4,5,6). These malaligned unaddressed structures can cause range of uro-gynaecological problems such as interstitial cystitis, dysuria (3), over active pelvic floor, pudendal neuropathy (5), dyspareunia, pre menstrual syndrome, dysmenorrhoea (4) and etc. Visceral reflex’s play a significant role in causing uro-gynaecological problems (7). Addressing these malaligned structures are essential for restoring the locomotory system to normal and to intervene with uro-gynaecological problems.

The knowledge in anatomy, biomechanics, pathomechanics, structural diagnosis, provoking special tests and appropriate manual therapy technique is essential for diagnosing and addressing PGD. In India diagnosis and manual physiotherapy intervention in pelvic girdle dysfunction is not taught in most of the educational institutions, most of the physiotherapy clinicians and students learning appropriate diagnosis and manual therapy interventions through manual therapy courses and workshops. The clinicians and students attending the courses and workshops are less in number.

The PGD among the Indian population is prevalent. The conservative management with medications and conventional physiotherapy with electrotherapy modalities, lumbo-sacral belts and exercises are not giving long term results. The pelvic girdle dysfunction need to be addressed by manual therapy to realign the malaligned structures, it should be followed by motor control and sensory motor retraining programme appropriately. Failing to realign themalaligned pelvic girdle structures with manual therapy approach can cause the “Dirty Half Dozen” in failed lower back syndrome as described by Greenman (1).

**Methodology & Materials**

To know about the knowledge of diagnosis and management in PGD among physiotherapy clinicians and students, self administered questionnaire had been designed and distributed to the participants who participated in the one day pre-conference workshop in Pelvic Girdle Dysfunction dogmas in diagnosis and approach on EYAN international conference in recent physiotherapy updates at SRM college of physiotherapy SRM institute of science and technology on 12th December 2018. Totally 36 participants attended the workshop and the survey using the self-administered questionnaire was conducted among this 36 participants. The questionnaire consisted 12 questions in 6 categories like 1) Willingness to update in PGD, 2) Knowledge of physiotherapy’s scope in PGD, 3) Knowledge about radio-diagnosis in PGD, 4) Knowledge about PGD, 5) Clinical decision making in PGD, 6) Knowledge about manual therapy in PGD. The questionnaire was given to the participants of workshop before the commencement of workshop and the participants were requested to fill it.
Analysis

1) Willingness to update in PGD

This category of question, is about the willingness to update their knowledge in PGD diagnosis and approach, for this 100% of participants had answered that they are willing to update their knowledge in PGD.

2) Knowledge of Physiotherapy’s scope in PGD

This category of question, is about the knowledge of the scope of physiotherapy in diagnosis and approach in PGD, for this 94% of participants answered that there is an scope for physiotherapy and 6% of participants answered there is no scope for physiotherapy in diagnosis and approach in PGD.
3) Knowledge about radio-diagnosis in PGD

This category of question is about the radiodiagnosis in PGD, for this 92% of participants answered radio-diagnosis is needed for diagnosing PGD and 8% of participants answered radio-diagnosis is not needed for diagnosing PGD.

4) Knowledge about PGD

This category of question is about the fundamental knowledge about the PGD, in this 28% of participants is having fundamental knowledge about PGD and 72% of participants is not having fundamental knowledge about PGD.
5) Clinical decision making in PGD

This category of question is about the knowledge of clinical decision making in PGD. In this 28% of participants is having knowledge of clinical decision making in PGD and 72% of participants is not having knowledge of clinical decision making in PGD.

6) Knowledge about manual therapy in PGD

This category of question is about the knowledge of manual therapy in addressing PGD among the participants. In this 6% of participants is having knowledge of manual therapy in PGD and 94% of the participants is not having knowledge of manual therapy in PGD.

Results

From the data obtained from the survey using the self-administered questionnaire. It was evident that most of the participants except those who attended previous manual therapy workshops or courses was not familiar with the concepts of PGD diagnosis and management approach. The results of the survey are 1) Willingness to update in PGD in this category 100% of participants are willing to update their knowledge in PGD, 2) Knowledge of PT scope in PGD in this 94% of participants is revealed there is a scope for PT in PGD and 6% of participants is revealed there is no scope for PT, 3) Knowledge about radio-diagnosis in PGD in this 92% of participants is
revealed radio-diagnosis is not needed to diagnose PGD remaining 8% is revealed radio-diagnosis is needed,4) Knowledge about PGD in this 28% of participants is having fundamental knowledge about PGD and 72% is not having it,5) Clinical decision making in PGD in this 28% of participants is having knowledge of clinical decision making remaining 72% is not having it,6) Knowledge about manual therapy in PGD in this 6% of participants is having manual therapy knowledge to address PGD and remaining 94% participants is not having it.

Discussion

The fundamental knowledge in PGD, clinical decision making and manual therapy knowledge in PGD, knowledge in these three categories among physiotherapy clinicians and students are lacking. There as an lack of knowledge in structural diagnosis of malaligned pelvic girdle structures, cluster of laslett to rule in sacroiliac joint dysfunction(9) and sacroiliac pain pattern. The lack of knowledge in Clinical Prediction Rule (CPR), sclerotomal pain pattern of sacroiliac joint, structural diagnosis of malaligned structures and in manual therapy is due to the comprehensive anatomy, complex biomechanics, less prevalence of evidence based practice. The orthopaedic manual physiotherapy is budding in India since past one decade. The orthopaedic manual physiotherapy practice is prevalent across the globe, but in India it is in infantile stage in grounds of practice, only few clinicians qualified in it and practising it as their full time clinical practice. The population of orthopaedic manual physiotherapist in India is scarce and very few institutions across India offering orthopaedic manual therapy education because of lack of adequate number of teachers in manual therapy, hence Indian physiotherapy clinician are not much into the practise of orthopaedic manual physiotherapy and for the same reason the physiotherapy students community are not much exposed to the orthopaedic manual physiotherapy. Moreover somatic dysfunctions is new to the Indian physiotherapy clinicians as well as students, this somatic model belongs to osteopathic medicine, Somatic dysfunction is impaired or altered function of related components of the body framework such as skeletal, arthrodial, and myofascial structures, and the related neurovascular and lymphatic elements(1). Structural diagnosis is evaluation of the musculoskeletal component with the objective of identifying the presence of somatic dysfunctions, it is an part of the physical examination. Most of the manual therapy techniques are borrowed from osteopathy (16). The one of the pioneer in Orthopedic Manual Therapy (OMT) FreddyM. Kaltenborn an physiotherapist, he was one of the founder of International federation of Manipulative Therapist (IFOMT) and now it was renamed as International Federation of Orthopaedic Manipulative Physicaltherapists (IFOMPT)(23) it is an sub group of World confederation of Physical Therapist (WCPT), he was the first clinician to integrate the theory and practice of orthopaedic manual medicine with osteopathy, his method of manual therapy techniques was from orthopedic medicine, osteopathy and his own techniques (19). The orthopaedic manual physiotherapist ‘deepak sebastian’(5), Timothy Flynn(20) and Christopher H. wise(6) had integrated somatic dysfunction models with physical therapy in their authored books. The physiotherapy clinician and students in US, Canada & Europe who learned osteopathic school of thought in manual therapy or mixture of traditional orthopaedic manual physiotherapy and osteopathy thoughts will be familiar with this somatic dysfunctions and structural diagnosis, for the physiotherapy clinicians and students in India it is new, Indian physiotherapy clinicians those who completed post graduation degree in physiotherapy and postgraduate physiotherapy students are familiar with Fryette’s law of spinal motion but they are not familiar with pelvic biomechanical models of Mitchels, Chicago and Stills(22). The American osteopathic text books advocates Mitchels biomechanical pelvic model, this model is commonly used by osteopathic practitioners in USA and Australia (24). These pelvic models are essential to address pelvic girdle dysfunctions, so it is necessary to include these models in physiotherapy curriculum along with the Fryette’s law which already exist in Indian physiotherapy postgraduate curriculum. Structural diagnosis based on somatic dysfunctions have to be introduced to Indian physiotherapy student community, especially when coming to pelvic girdle, because somatic dysfunctional model entails these structures dysfunctions detailedly than other school of manual therapy thoughts. Emphasis on sclerotomal pain patterns in physiotherapy curriculum from undergraduate level have to be made mandatory to make understand the pain patterns of facet joints and sacroiliac joint(18). Introduction of
orthopaedic cluster tests\(^9,11\) and decision making tools such as Clinical Prediction Rule (CPR)\(^{10,11}\) to the indian physiotherapy clinicians and students must be made to inculcate the evidence based practice. More over including the concepts of diagnosis and manual therapy management of PGD in the books of orthopaedics,obstretrics& gynaecology ,sports physiotherapy are essential it will benefit the indian clinicians and student community.

**Conclusion**

Introducing the concepts in diagnosis and management of pelvic girdle dysfunction to the indian physiotherapy clinicians and students is essential to serve the patient community. Thefundamental knowledge of pelvic musculoskeletal structures and its mechanics is mandatory. The pelvic girdle dysfunctions are precursors of various orthopaedic and uro-gynaecological problem, The pelvic girdle dysfunctions causes the affected person to cripple and it create annoyance. The diagnosis and management of pelvic girdle dysfunction is not confined to orthopaedic manual physiotherapy speciality alone, the orthopaedic manual physiotherapy is tailored with the obstretics and gynaecological physiotherapy,sports physiotherapy and biomechanics specialization in physiotherapy. The pelvic girdle dysfunction is predominantly an orthopaedic problem, but other physiotherapy specialities has a equal scope in diagnosing and addressing the dysfunction , for an instance obstretics and gynaecological physiotherapist is having spectrum of scope in addressing Pregnancy-related Pelvic Girdle Pain (PPGP)\(^17\), this PPGP can be addressed by realigning the malaligned pelvic girdle structures withimplusive techniques (thrust techniques) and non-impulsive techniques (non-thrust techniques) such as articular technique and muscle energy technique\(^7,12,13,14\) and range of urogynaecological problem can be addressed with manual approach to pelvic girdle structures, the articular dysfunction (malalignment) in sacroiliac joint may aggravate the source of myofascial trigger points in pelvic floor muscles\(^15\). In sports physiotherapy ranging from groin pains,gluteul muscle inhibition\(^1\) and etc can be addressed.In biomechanics there is lot of scope in biomechanically analysis of pelvic girdle structures. There is a spectrum of scope in these physiotherapy specialities in diagnosing, addressing and researching in pelvic girdle structures. Includingrecent evidence based concepts in diagnosis and management of pelvic girdle structures in physiotherapy curriculum will help the student community as well as clinicians and it increases the cognizance of pelvic girdle dysfunctionsamong them to serve the patient population.

**Conflicts of Interest:** The author does not have any conflicts of interest

**Source of Funding – Self**

**Conflict of Interest:** Nil

**Acknowledgement:** Authors expresses their appreciations and thanks to Mohana krishnanjagadevanPT, PhD physiotherapist JIPMER puducherry India and Bhanumathi mohanakrishnan PT, physiotherapist JIPMER puducherry India, for assisting in manuscript preparation and statistical analysis.

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Patient Safety Program in a Hospital with Reference to Costs Associated with Patient Falls

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Abstract

Background: Patient safety is an emerging field in healthcare that has indirectly or directly affected all stakeholders of healthcare delivery system in the last decade. World Health Organization guidelines for adverse event reporting and learning systems, 2005 describes adverse events as “an injury related to medical management in contrast to complications of disease.” These adverse events can be preventable or non-preventable. Inpatient falls presents a serious challenge to the safety and quality of patients in hospitals. Falls are not harmless, they delays in functional recovery and prolonged hospitalization that can cause patients to suffer. The study was conducted to estimate the incidence and major reasons for falls.

Methods: Retrospectively, case records from January 2016 to September 2018 of all the inpatients were researched for patient fall incidents, reported to the committee. The committee findings noted and the costs were estimated in terms of cost to the patients, and costs to the hospital.

Results: 118 incidents of patient fall happened in study period. 3(2.6%) of them experienced two or more falls during stay, 115 (97.4%) fell once. 5 patients were morbidly obese. Costs to the hospital on each patient was Rs. 5412/-, not levied on patients.

Conclusion: The number of fall incidents and falls reporting system have been improved by early detection of reasons by conducting root cause analysis.

Keywords: Patient safety, Indian Patient falls, Obesity, Consciousness, Facilities Management.

Introduction

The healthcare organizations in developed world have been swaying under the effect of the adverse public opinion generated as a result of a number of studies on medical errors. Patient safety is an emerging field in healthcare that has indirectly or directly affected all stakeholders of healthcare delivery system in the last decade. Inpatient falls presents a serious challenge to the safety and quality of patients in hospitals. 6.63 falls per 1000 occupied patient days on an average equates to more than 1700 falls every year in an 800 bedded hospital general hospital in recent audited data. The estimated falls rate per 1,000 bed days, a provider with 800 beds will have approximately 1,500 falls at a cost of £3.9 million. “Patient safety is the absence of preventable harm to a patient during the process of healthcare and reduction of risk of unnecessary harm associated with health care to an acceptable minimum. More than 250,000 are recorded annually as well as they are the most frequently reported safety incident. “An average of 6.63 falls per 1,000 occupied bed days (OBDs), which equates to more than 1,700 falls every year in an 800-bed general hospital at current bed occupancy rates, has been exhibited by recent audit data”. Physical injury and fractures occur in 1–3% as a result of some 30-50% of falls. The estimated falls rate per 1,000 bed days, a provider with 800 beds will have approximately 1,500 falls at a cost of £3.9 million. “A patient fall is an unplanned descent to the floor (or extension of the floor, e.g., trash can or other equipment) with or without injury to the patient, and occurs on an eligible reporting nursing
unit”. Patient falls are designated as one of the “National Patient Safety Goals” by “The Joint Commission on accreditation of Healthcare Organizations.”

The causes of falls can be classified under: 1) Staff Factors: Incomplete assessment of patient and failure to elicit the history of falls. One of the strongest predictor of falls is - Inadequate staff, Insufficient training. 2) Patient Factors: Lack of communication regarding previous history of falls by patient or relatives, Morbid conditions of patient like poor vision, obesity, diabetes, hypertension, severe anaemia etc., Type of medications, Patients on sedatives, Anti- Diabetic medications, 3) Organizational: Lack of policy to avoid falls.

“Quality Indicators (QIs) are standardized, evidence-based measures of health care quality that can be used with readily available hospital inpatient administrative data to measure and track clinical performance and outcomes”, which highlight potential quality improvement areas and help in track changes over time. According to Continuous Quality Improvement chapter of National Accreditation Board for Hospitals and Healthcare providers, a CQI standard 4 and objective element b says, Monitoring includes risk management; incidence of falls is one of the key performance indicators which the organization should monitor.

Through this study, we found out the incidence and major reasons for falls along with it estimating an approximate cost incurred due to each patient fall using different statistical methods. The hospital has a total of 10 blocks.

**Aim:** To assess patient falls and the cost associated with it in tertiary care teaching hospital, with Objectives of: Estimating the incidence and the reasons of patient falls, estimating the cost involved and potential savings to the hospital from reducing those falls, suggesting recommendations for downgrading such falls

**Methodology**

A cross sectional, prospective and retrospective study was performed, wherein case records were reviewed from January 2016 to September 2018 of all the inpatients who were admitted in the hospital. Costing estimation was done. The hospital uses incident reporting software where all incidents are uploaded and reported.

**Study design:** After obtaining the clearance from Institutional Ethical Committee, the Details of In-patients admitted in casualty, Operation Theatre, Intensive Care Units and Surgical Wards having incidence of falls, reporting to Safety & Sentinel Committee of the hospital, were collected. The details of patients like UHID (Universal Hospital Identity number), Time, Location, where the incident happened, Reasons of fall, fall risk assessment score, dependency of the patient on nursing staff all these parameters were obtained through the incident management software and medical records of patients. All patients with incidence of fall have been included, patients without falls have been excluded for the study. **Study period:** Six months durations, November 2018 to April 2019. **Study population:** In patients falls reported and documented in a tertiary care hospital from January 2016 to September 2018. **Study tool:** A checklist for data collection was framed and records from incident management software and medical records were obtained. The incidence of falls was collected and calculated by a formula given by National Accreditation Board of Hospitals and Healthcare Providers. The formula used was: (Number of patient fall reported in a period / Total patient days in that period) x 1000.

**Study Setting:** The hospital is a tertiary care teaching hospital with 2000 beds with all clinical and non-clinical departments. The average bed occupancy of the hospital is 85%, with 80,000 people getting admitted per year. The hospital had admitted 200,000 patients during the January 2016 to September 2018. The wards are divided into 400 beds of single rooms with attached bathrooms, 800 semi-private rooms with sharing bathrooms, 800 of general ward beds with common bathrooms. All the floors in corridors, ramps, bathrooms have non-skidding tiles, mosaic tiles etc. The hospital is certified with National accreditation board for hospitals and Healthcare providers (NABH).

**Results**

A total of 118 patients experienced fall during the study period. These patients accounted for 121 incidents of falls, as 3 (2.6%) of them experienced two or more falls during their admission period of single admission, while rest 115 (97.4%) fell once during single admission
episode. 40 patients were obese, with 10 people mild obese, 25 with moderate obesity and 5 people were morbid obese. For our estimation of costing purpose, the first falls of the patients was only accounted as second and third fall may become a bias for patient attributions as multiple fallers tend to repeat the location and type of fall on subsequential falls. The eldest patient who fell had an age of 52, years 2 months and the average age of patients ranging around 40-60 years. Incidence of falls was 0.034, 0.06 and 0.13 for the years 2016, 2017 and September 2018 respectively, which is depicted in Chart 1. The maximum falls happened during March (n=13) month of 2018, and the rate was 0.27 per 1000 patient days and next in August (n=12) 2018. The frequency of falls range from 13 a month to zero falls reported during February, June, July, December 2016, and April 2017.

The reasons identified by the floor nursing staff were, falling from bed while raising was the reason in 25 incidents (21% of cases), as they were not using side railings which were provided. Chart 2 describes all the reasons. A pamphlet is given to all inpatients regarding do’s and don’ts to avoid falls. The patients with hypoglycaemia was the common reason for giddiness and blackout. The patients slipping in bathroom was next common reason. Side railings have been provided next to shower, western toilets. About (20/118; 16%) falls happened while the patient was using the bathroom. Generally, falling in bathrooms have been reported in early morning timings between 4 AM to 7 AM, when patients do not wake up their attendant. 42.5% of falls were seen during Nursing night shift timings, 38% were during the morning shifts (7 AM to 2 PM), 20% were in evening shifts (2 PM to 9 PM). The floor was wet for 3 (2.54%) of the falls, we started making it mandatory for environmental health workers to use yellow sign boards to indicate, people walking around that area, that cleaning is in progress. Issues with chairs and stools or furniture malfunction contributed to 12.7 % and 8.47% respectively. 2 patients had fallen in acute areas, rest of all patients the incidents were reported from long stay ward areas. Block 2 of the hospital where general medicine and surgery wards are located reported more number of falls whereas block 4 with special category had least.
Chart 2: Environmental Circumstances of patient falls in this hospital:

Overall, 80% of the 118 falls resulted in certain type of injury, as shown in Chart 3. 4 (3.2%) of them had stroke or cardiac event leading to fall and subsequent death and 24 of the 122 incidents (20.3%) had fractures while many of them suffered moderate injury like wound and laceration (15.25%), bruise (5.9%) while few cases had sprain (3.38%), and dislocations.

Chart 3: Type of injuries recorded after the fall incident:
Every fall was associated with additional hospital costs which included investigation and consultation charges while few had to undergo intervention procedure and medication costs. For the purpose of study, as shown in Chart 4, a box and whisker’s plot was created as the data for the cost was highly skewed. Therefore, Procedural and medication costs were calculated together which estimated to be around Rs. 4407 (Chart 4 B), while the Investigation and consultation costs were Rs. 4140 (Chart 4 A). Approximately Rs. 5412.3 was the total cost incurred from each patient fall (4 C). Whenever a patient fall happens the care given to patient may vary according to the seriousness of fall. In cases of No harm; vitals were checked by the duty postgraduate doctor, simple first aid was given and in cases of requirement, analgesics were given. The common analgesics given in the study hospital were Voveron gel and tablets, Hifenac - P were given while in Moderate injury cases: orthopaedics, physiotherapy or according to disease ailment consultation was provided to the patient. Common investigations done were X-Ray, MRI and CT-Scan for head or limb injury cases. In cases of sprain and fracture (n=22) arm sling and splint were provided to the patient.

Needed cases open reduction and internal fixation, close reduction and Tension band wiring and K-wire fixation was also done and patient had to stay for 5 days more.

Discussion
“Falls are not harmless, along with psychological sequelae that lead to loss of trust, delays in functional recovery and prolonged hospitalization that can cause long-term patients to suffer. Falls are not true accidents, however, and there is attestation that a coordinated multidisciplinary clinical team approach can reduce their incidence”. The nurse patient ratio for general wards can be optimised as critical falls were happening in these wards. The current ratio being 1:3 per 24 hours duration, 1:7 beds per shift, it can be brought down to 1:5 beds per shit. This would improve public relations, constant communication to the patient attendants and the
patients about precautions to be taken while raising from the beds, using washrooms. In one study done in similar setting, overcrowding in wards is seen as single most individual factor perceived by all study participants potentially leading to violence and injuries to patients. The injuries sustained range from head to limb injuries.\textsuperscript{9} Bed alarms and other protective measures in special wards can be provided. Inpatients should have an easy access to call bells and bed alarms can be provided as simple safety measure. Use of lower size beds and bed rails. For the morbid and moderate obese patients, this change will lead to reduction in number of falls. Staff orientation: Awareness and training sessions were taken for nurses to follow extra precautionary measures (there should be standardisation of practice and application of interventions). Low back injuries are the leading occupational health problems affecting healthcare workers and are increasing among nurses and nurses’ assistants. The primary risk factor for low back disorders among nursing personnel is lifting and transferring of patients. During the transfer of patients, nurses of origin ward and receiving wards should make ward helpers to reduce the risks of patient falling from stretchers.\textsuperscript{10} Risk analysis at the time of hospitalisation: Fall Risk Assessment score is noted for all the in-patients during the time of admission can be improvised as in many medical records it’s not filled completely. As part of building management, usage of non-skid tiles and installation of grab bars at two different levels for wheel chair bound people and normal patients, where appropriate and use of well illuminated lighting in the sanitary areas has been implemented through patient safety committee. The patients should be constantly accompanied by either patient attendant or the nursing assistants. None of the patients who fell in this hospital, required any further rehabilitative services. No physiotherapy requirements were noted during the study, hence, they are not included in the costs. We included movement analysis tests as part of training program to Nurses, who will in turn make patients aware of the situations and symptoms which will aggravate the fall incident. Once patients are aware, their fall rates at home surroundings will get mitigated and re-admission rates will reduce to hospital and burden on healthcare delivery system gets reduced.

**Conclusion**

The influence of falls on Hospitalized patients should be defined as well as the areas where hospital administrators need to keep an eye are potentially effective interventions. Once a problem is suspected, one may be tempted to move directly to intervene. Initiative from the leadership to motivate hospital staff to reduce/prevent the falls from happening can be implemented. Multifaceted and multidisciplinary approach is required for prevention of falls inpatient settings. Single definitive factor is not silver bulleted in preventing falls.

**Conflict of Interest:** No.

**Sources of Funding:** Self, Acknowledgement: Manipal Academy of Higher Education.

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Physical Therapy Rehabilitation and Care in Post Operative Trans-Tibial Amputation Patient

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Abstract

Introduction: The case study is focused on the treatment of an individual with a residual limb after a transtibial amputation. The main goal is to help provide a greater understanding of how to treat the residual limb after transtibial amputation.

Case Description: The case study focuses on a 65 year old female who recently underwent a transtibial amputation of the left lower extremity because of chondrosarcoma of distal tibia. The patient was mesomorphic and had a history of tumor. Physical therapy provided care in ways including wound care, therapeutic exercise, gait training and prosthetic fitting and training.

Conclusion: The patient progressed well because of her motivation to continue physical therapy and the proper management. After much hard work, by both the patient and the therapist, the patient was able to ambulate house distances without assistance. For the patient’s age, it was remarkable to be able to progress to the current functional status which she attained.

Key words: Transtibial, amputation, treatment, rehabilitation.

Introduction

The case of a 65 year old female is reported, who underwent below knee amputation following the diagnosis of chondrosarcoma in lateral malleolus of left fibula. Patients that need care after amputation have been a main stay of the medical community for many years, but new research and treatment methods need to be pioneered for the comfort and functionality of amputees. A lot of research has been done recently with the use of 3D printers and their application to the amputee treatment process but there are still many areas of treatment that need improvement, especially surgical procedure, wound healing, prosthetic fitting and prosthetic gait training. The primary aim of the physiotherapy rehabilitation in case of amputation is to improve the community mobility of amputees. To attain this goal, it is necessary to design individual rehabilitation protocols specifically for each patient depending upon his or her functional ability, societal requirements, and motivation. Initially when transtibial prosthesis is fit, the patient finds it difficult to regain his function and mobility.

Maintenance of ambulation with the use of a prosthetic limb is seen as an important factor associated with preserving independence. Normally, patients who require a transtibial amputation are older in age and there is a high chance for them to suffer from clinically depressed after the surgical procedure. So, it is necessary for the physical therapist to be caring, competent, and have an understanding of the patient’s emotions. Also critical are the knowledge and skills for providing the patient with the best treatment possible.(1)

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The patient normally starts pre-prosthetic physical therapy immediately after the transtibial amputation. Pre-prosthetic rehabilitation includes upper and lower body strengthening exercises while maintaining range of motion in the lower extremity. This can be difficult as a result of changes in the leg musculature during the amputation surgery, which can either be from being cleaved or atrophied from inactivity. The physical therapist will perform desensitizing the patient’s residual limb by using skin rolling, tapotement, and soft tissue mobilizations.\(^{(2)}\)

**Patient Information:** A 65 years old female farmer with normal body mass index and right hand dominance complained of pain and burning sensation and swelling at the lateral malleolus which later spread to the whole foot. With these complaints, she visited AVBRH where radiological investigations were done along with other investigations. She was diagnosed with chondrosarcoma and planned for below knee amputation, i.e., transtibial amputation so as to control the spread of the pain and swelling due to the tumor. She had a history of tobacco chewing since 20 years with no other addictions. She had a past history of hospitalization for the same complaint 1 year earlier to the surgery. At that time, excision biopsy was performed but the patient and her family refused to undergo operative treatment. She lives in the ground floor of her 2 storey house, so that she isn’t required to climb stairs. She has her 2 sons and 2 daughter-in-laws to take care of her. Transtibial amputation was performed on 22\(^{nd}\) September 2019. Post-operatively, she was referred to physiotherapy department with the complaint of pain at the suturing site and difficulty in performing functional activities.

**Clinical findings:**

On post-operative day 1 after the amputation, the patient’s heart rate was 74 bpm, respiratory rate was 18 breaths per minute, blood pressure was 110/70 mmHg. The patient was alert and oriented with time, place and person. The patient’s overall posture was assessed and no abnormalities were detected besides loss of her left leg. Her residual limb was warm to the touch and swollen, showing cardinal signs of inflammation. The residual limb measured 34 centimeters in girth at the level of 15 centimeters below the patella and her wound was closed with sutures. Since it takes time to know how the incision site will seal or if it will seal completely at all, hence monitoring was done regularly. Pain stated by the patient was 4/10 on NPRS scale. Measurements of the active range of motion (ROM) using goniometer at the hip joint were: 100\(^{\circ}\) of hip flexion, 30\(^{\circ}\) of hip extension, 25\(^{\circ}\) of hip adduction and 40\(^{\circ}\) of hip abduction. She showed an extension lag of 15\(^{\circ}\). The patient was unable to perform flexion beyond 110\(^{\circ}\).

Manual muscle tests for right lower extremity were performed. Her strength was 3+/5 for hip flexion, 4+/5 for knee extension, 5/5 for knee flexion, 3/5 for dorsiflexion and 4/5 for plantar flexion. Manual muscle tests of the left lower extremity were deferred due to pain. The Functional Independence Measure (FIM) was the primary tool used to evaluate changes in areas such as ambulation, bed mobility and balance for the patient because of its reliability and validity. The patient was unable to go from sit to stand independently. She required max assist of 1 or a moderated assist of 2 to stand, making her a FIM Level 2. The patient required minimal assistance with all bed mobility skills making her a FIM Level 4. Her balance in the seated position was reduced; however, she did not require assist to maintain an upright sitting posture. She needed supervision for safety while sitting, making her a FIM Level 5. Balance in standing was not tested.

The patient’s upper extremity strength and range of motion were adequate to carry out the activities of daily living. Her lower extremity strength in her uninvolved limb was functional as evaluated by MMT and she had no previous history of weakness in the same. The main problems as per standard International Classification of Functioning, Disability and Health (ICF) model were: decreased strength of both involved and uninvolved lower extremity, decreased ROM in extension of involved hip and bilateral upper extremity, decreased balance/postural control in both sitting and standing, decreased endurance because of bed rest and loss of limb, loss of ankle joint which makes ambulation with prosthesis difficult.

**Therapeutic intervention:**

Intervention consisted of two phases.

In the first phase, pre-prosthetic training and emphasized range of motion stretching, especially
hip and knee flexion stretching were done. Strength training was performed for her residual limb and sound limb, particularly strengthening the hip abductors of her residual limb to assist with maintaining proper stability in gait. Scar and soft tissue mobility were performed on the patient’s residual limb for gait training and weight bearing in the prosthesis, ensuring proper mobility of the skin on the residual limb stump and reducing pain. In addition, transfer training, wheelchair negotiation training and standing balance training were performed. (3)

In the second phase of therapy, donning and doffing training of the patient’s lower extremity prosthesis was performed along with application of socks. Weight shift training was practiced while standing in the prosthesis in a lateral and diagonal fashion. More challenging exercises were added progressively to increase the patient’s single leg stance ability. (4)

Gait training was initiated within the parallel bars, progressing to walker and then cane as an assistive device. Stairs, ramps, outdoor gait and functional training such as lifting heavy items off the floor, reaching for higher shelves, etc were also performed. (5)

Follow up and outcome:

By the end of the sessions, she was able to walk without any assistance and was independent in mobility. The patient came for physical therapy with motivation and was readily willing to do whatever she was asked. She was taught home exercise programmes, which she performed sincerely and came for follow up weekly. She started with her full Functional activities and activities of daily living within 4 months. The patient’s psychological well being was also a positive factor which helped the treatment plan to work with the time of recovery estimated for her.

Discussion and Conclusion

This patient is a great example of what the prognosis after an amputation should look like. Motivation plays a huge role in the improving the outcomes of any treatment. This patient’s family’s and her own will to recover faster helped play a big part. As the patient suffered for a year prior to the elective procedure to ampute, she was mentally ready to face all the challenges in the path of recovery. She complied with the physiotherapist better due to her past references with pain. All these factors counteracted the barrier of age and easy accessibility of the physiotherapy. (6)

The patient opted for no prosthetic fitting due to her poor economic condition. This did not hamper much of her life as she was a home maker with her daughter-in-laws for support. But would that be a case where the social life and economic necessity in reference to job would have come, then counseling for a proper prosthetic fitting and budgeting the cost of the prosthetics be the agendas in the therapist goals. That would lead to prosthetic training and ergonomic transformations in home and workplace. Transportation facilities and daily commute with public transport would also be important (7)

Ethical Clearance: The institution Ethics committee clearance is obtained.

Conflict of Interest: Nil.

Funding Support: None.

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Impact of Workstation Exercise and Ergonomic Exercise on Nursing Population – A Randomized Clinical Trial Research Protocol

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Abstract

Nursing workers represent a significant proportion of the hospital workforce. In recent times, focus is put on increasing efficacy with reducing the concurrent risks in this population. Many researches imply on including workplace modifications and various exercises to reduce the number of absenteeism and heighten the quality in work. As low back ache is the most prevalent, this study targets on determining the effects of workstation exercise and ergonomic intervention on it.

Purpose - Nursing staff is the base of primary healthcare provision in the Indian background. The most common reason for early retirement, sickness absenteeism, and decline in working population in nurses is low back pain. With proper evidence, the efficacy of workstation activities in nursing staff will mitigate or eliminate this issue in the area of healthcare.

Methods: There will be 3 groups, workstation exercise group, ergonomic modification group and a control group each consisting of 40 patients selected from a fixed criterion. They will be graded with a QOL scale and a pain scale during the start of treatment and after 2 months to compare the results and determine which therapy gets better results.

Results: Statistical analysis will be done after the study’s completed by use of concise and inferential tests use Student’s distribution t test on single and combined analysis, one-way Analysis of Variance (F) ANOVA test and Multiple Comparison: Turkey Test and analytical tools is SPSS24.0 version and Graph Pad Prism 6.0 version and p<0.05 is known as value point.

Conclusions: Both the ergonomic training and workstation exercises will result in reduction of low back pain with workstation exercises proving more effective. The Publication will be done after conducting the study and obtaining the results through the statistical analysis.

Key Words: Workstation Exercises, Ergonomic Intervention, Quality of Life, Low Back Pain, Nursing Staff, Physiotherapy Approach.

Background

Musculo skeletal conditions are the utmost public wellness issue.¹Those that requires medical condition attention, a difficult issue to tackle with many professional groups, such as the nursing staff. Even though the nurses are trained to administer to such health issues of others, they neglect their own well being for the
sake of reduction of taking leaves and better job caliber. Historically, back pain has become a more common complaint, and most of the health care practitioners are at the greatest risk. Many researchers have become a witness to this condition in their trials.2

Community of low back pain (LBP) is highly prevalent, and it has been explored in much research.3 Findings put it on record that perhaps a multitude of factors relate toward low back pain globally. Some factors include age, genes, body mass index, comportment, style of living, vocational jeopardy, and many others or idiopathic in nature. However the etiology has not yet been known. Thus, intervention as well as preventive strategies was found to lack of documented feasibility on the basis of etiology.4

The ergonomic changes to counter act or reduce the pain from low back pain are prominent. The hospital attendants are thoroughly well versed with these. Even though ergonomic modifications have been shown in certain research to also be moderately successful in reducing musculo skeletal disorders, they are very expensive which would be an important factor particularly in developing and undeveloped countries. In such times, the work place training comes in handy. It is relevantly cost effective and also helps to save time and money in recurrent process of modulating and refurbishing of the wards or clinics. It can be worked as it is, to build more support by cost efficiency. In other words, work place work outs save the time and money to start or initiate the treatment program to relieve low back ache.

When determining progression of the disease, the diagnosis, as well as the control of any of the musculo skeletal disorders, Q O L scales are now being used. As widely relevant Q O L is defined as a term that reflects individual responses to different effects of disease on everyday life, affecting to what level real fulfillment should be attained. Measurement of Q O L is detected in recent clinical trials as a significant add-on to achieving therapeutic effectiveness for the beneficial change. Low back discomfort is actually a major deterrent to Q O L and the Q O L scores co related with low back pain and other disabilities.6

Visual Analog Scale is a validated and utmost used in all the musculoskeletal, neurological or other conditions. It does not diversify pain in various components and hence is reliable in pre tests, post tests and follow ups. Language barrier or educational intelligence and other factors do not hamper the outcomes of this pain rating scale.

Through this research, we aim at estimating and contrasting the influence of ergonomic alteration and work place exercises on low back pain in a crowd of nursing health care providers.

**Objective**

1. Assessment of low back pain
2. Analysis of effectiveness of ergonomic changes in low back pain
3. Analysis of effectiveness of workstation exercises in low back pain
4. Comparison of the effects to analyze superior treatment method

**Material and Method**

The study is set at Datta Meghe Institute of Medical Sciences, Sawangi (Meghe).

The Study design is randomized clinical trial in an intervention group of nursing personnel. Participants will be assigned their groups randomly or by chance.

Study setting - Ravi Nair Physiotherapy College, A V B R H

Sample type – randomized sampling

Sample size – 120

The sample size formulae used are as follows

\[ n_i = \left( \frac{\sigma_i^2 + \sigma_2^2}{\kappa} \right) \left( Z_{1-\alpha/2} + Z_{1-\beta} \right)^2 / \Delta^2 \]

The notations of formulae are

- \( n_i \) = sample size of Group 1
- \( n_2 \) = sample size of Group 2
- \( \sigma_i \) = standard deviation of Group 1
- \( \sigma_2 \) = standard deviation of Group 2
- \( \Delta \) = difference in group means
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\[ K = \text{ratio of } \frac{n_2}{n_1} \]

\[ Z_{1-\alpha/2} = \text{two sided Z value (e.g. } Z = 1.96 \text{ for 95\% confidence interval)} \]

\[ Z_{1-\beta} = \text{power} \]

- Group A with 40 participants under ergonomic intervention group.
- Group B with 40 participants under workstation exercise group.
- Group C with 40 participants under control group.

Sampling will be allotted randomly. The division of the groups would be on the basis of the time and number of subjects received. The first subject will be allotted Group A, the second subject will be allotted Group B, the third subject will be allotted Group C. the Fourth with Group A and continued henceforth. The chronology of the patient list will be solely dependent upon the time and date of assessment rather than the Alphabetical order or age or other criterions.

Study duration - 6 months

**INCLUSION CRITERIA**

- Patients willing to participate.
- Permanent nursing staff.
- Age group 18-50 years.
- Diagnosed with low back pain.
- Pain persists more than 1 month.
- Both genders.

**EXCLUSION CRITERIA**

- Patients not willing to participate.
- Patients not willing to continue treatment for long duration
- Individuals with other musculoskeletal and neurological issues.
- Age group above 50 years.
- Individuals with chronic low back pain.
- Interns and temporary job nurses
- Recent parents with infants and children
- Other chronic conditions.
- Chief cause of pain irrelevant to the nursing chores

**MATERIALS**

- Printed consent forms for participants.
- Printed sheet of Visual analog scale.
- Printed sheet of WHOQOL BREF scale.
- Ergonomic advice charts.
- Thera bands.
- Lumbar corsets.

**DEPENDENT VARIABLES**

- Visual Analog scale
- WHOQOL BREF

**INDEPENDENT VARIABLES**

- Workstation exercises.
- Ergonomic intervention.
- Lumbar corset.

**PARAMETERS/ OUTCOME MEASURES:**

- Visual analogue scale for intensity of pain measurement
  - Reliability: 90\%, Validity: 76\%-84\%
- WHOQOL BREF scale for functional disability and quality of life assessment
  - Reliability: 76\%-80\%
  - Validity: physical domain- 67\% , Psychological domain- 78\% , Social domain- 74\% , Environmental domain- 86\%

**PROCEDURE**

The institution ethics committee clearance will be obtained 120 participants will be selected, nurses
who are diagnosed with low Back pain from AVBRH hospital as per the criteria. The informed consent will be obtained. The participants will be explained about the type of study in their own language. They will be divided into 3 groups and each group would be explained the steps of intervention. They will be provided with visual analog scale and WHOQOL BREF scale. Pre-treatment pain and overall quality of life would be calculated. Group A will be educated and facilitated with ergonomic interventions and charts will be given depicting the same. Group B will be taught stretching and strengthening exercises to be done within scheduled breaks during the work hours. Group C will be the control group that will be given lumbar corsets to use. This group will serve as the standard for comparison for Group A and Group B. After two months of treatment, follow up of all the groups will be taken with the same scales and thus will be compared. The data collected will then be analyzed statically.

Hypothesis: The Group A of ergonomic interventions will yield positive results wherein the mean of observations of the Group B of workstations exercises will be more inclined to the value of 1.00 levels. Group C will be inclined to 0.05 re 0.01 level of null hypothesis.

The independent variables of this study will not have co relational analysis with the dependent variables. This will help the study to move forward to the rank order co relation in the specific statistical analysis.

**Data Analysis**

Statistical analysis will be done after the study is completed by use of concise and inferential tests use Student’s distribution t test on single and combined analysis, one-way ANOVA and Multiple

Comparison: Turkey Test and analytical tools is SPSS24.0 version and Graph Pad Prism 6.0 version and p<0.05 is known as value point.

The t test is used to determine the statistical significance in small sample observations for near correct conclusions. It rectifies the difference in the curve of small sample distribution when referred to the reliable large sample distribution curve. Many t table list values at various degrees of freedom of rejection of the null hypothesis are at 0.05 and the 0.01 level of significance.

Analysis of Variance (F) ANOVA test is a useful method to pinpoint if the sources of more than the two simple random samplings are sufficiently close for sampling measurement variances and errors to be related. It raises the question if the sample means differ from their own sample means (under variability of the category selected). The significance of the ‘F’ - ratio is found in ‘F’ - tables which indicate the values necessary to reject the null hypothesis at the 0.05 or the 0.01 levels.

If the global null hypothesis Ho is dismissed, the researcher’s greatest interest is to know how the meaning of t treatments varies. If the global null hypothesis Ho is denied, the researcher’s greatest interest is to know how the meaning of t treatments varies.

For each pair of means the Turkey test decides if they are substantially different and is based on a family error rate for comparisons k = t (t-1)/2. The procedure is to test the hypotheses: Ho: μi = μi, vs. Ho: μi = μi, I = 1, t, and Ho are rejected at a meaning level if

\[ m_i - m_{i'} > q s \sqrt{1/r_i} \] or \[ m_i - m_{i'} > q s \sqrt{1/2(1/r_i + 1/r_{i'})} \]

Where mi and mi’ are estimates of the means and ri and ri’ are the number of replicates of the treatments I and i’ and q = qt, a is the value of the student range with t means, n degrees of freedom associated with s2, the Residual Mean Square.

Prism is a versatile blend of biostatistics, curve fitting (nonlinear and empirical regression) grappling with a robust plan. It may report on intervals of confidence of best-fit parameters as asymmetric ranges (likelihood of profiles), which are far more precise than normal intervals symmetrical. Even it will automatically interpolate unknown values from default curves (I.e., the analysis of RIA data), the comparison of two fits equations using or the Knowledge Criteria for Akaike (AIC), plot residuals, differentially classify outliers weight data points, the normality test residuals and lots more.

As the Sample size is significantly smaller and there are more than two samples, the sampling error will be larger with concerns of the graph of distribution of the means. Altogether in random sampling methods, the
window of error is more than systematic, cluster or convenience sampling. Thus for precise analysis and conclusion of the study, we will use more than one statistical tool. The values generated will justifiably be helpful in rejection of the null hypothesis.

**Expected Result**

After completing study, result will be calculated by statistical analysis and will be prepared and published in the form of a research paper.

**Discussion**

At the end of the study duration, both the groups’ observations would manifest a cogent that both the work place exercises and the convenient ergonomic mediation work wonders on the nurses suffering from low back ache.

The consistency of results after follow up in four domains of the Q O L scale is not pinned down in all the three groups. The physical and psychological domains show highest average in satisfaction whereas the social and environmental domains don’t show much equivalency. This might be due to the level of work stress which was not well accounted for during the period when study was conducted. The longer version of the Q O L can also be applied in further studies for precision.

Difference of findings with the help of Numerical Pain Rating Scale and Visual Analog Scale is almost negligible. Although the Numerical Pain Rating Scale might have more precise value in follow ups. Other new found Pain Rating Scales might also be verified and used for the purpose of fulfillment of the agenda. Although the researches on the more modern approaches to narrow down the pain criterions and denounce its measurement are continuously advancing.

In this study, effects of ergonomic training and work place exercises were evaluated separately. Sahu A, Naqvi WM explained thoroughly the effects and influence exercising does on the human body. It covers all the physical, psychological, pathological, social and moreover importantly environmental and mental aspects in which the exercises help our body. The impact would increase with both the manipulations combined for low back pain. Further studies may involve another group with this type of an intervention. Both the exercises and the ergonomic rules would be compared with a more convenient control group to estimate the amount of recovery from the pain.

The stretching and strengthening exercises both fall under the work station exercises. They both have different effects on the muscles of the lower back. At such times, clinical reasoning for the etiology should be considered before chalking the plan of treatment. This can be achieved with the help of a thorough study of the biomechanics of the lower thoracic, lumbar, sacral and sacroiliac joints.

The inclusion criteria and the exclusion criteria are more precise regarding this study setting. Although every study setting might include more exclusion criteria based on the demographical aspects, geographical aspects, medical characteristics and / or any other external characteristics. With the changes in the goals and objectives of the study, the inclusion and exclusion criteria will change drastically. After the completion of sampling process, additional data of the participant can also compromise the analysis and make it inaccurate or misleading or biased.

Intervention control in all the trials proves as a facilitator to provide and also to compare the statistical analysis. The control groups have a tendency of placebo effects degrading the reliability and validity of the experiment. Thus, cautious selection of the control group and activity allotment is requisite. Pharmacological drugs, yoga, hydro collator pack are some of the activities applicable in control groups for such practice.

**Conclusion**

The Publication will be done after conducting the study and obtaining the results through the statistical analysis. Both the ergonomic training and workstation exercises will result in reduction of low back pain with workstation exercises proving more effective.

Null hypothesis - Follow up results of low back pain will be same for all 3 groups.

**Ethical Clearance**: Clearance will be sought from the institutional ethics committee.


Source of Funding: Self
Conflict of Interest: None

References


Correlation of Matrix Metalloproteinase-2 and p21 Expressions with Capsular Invasion of Thymoma AB

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Abstract

Thymomas are the common neoplasms of mediastinum, and type AB is the most frequent case. All thymomas are potential for invasion to adjacent tissues and considered malignant. Capsular invasion is one of its prognostic indicators. Matrix metalloproteinase-2 (MMP-2) is a family of proteinases that has the ability to degrade a component of extracellular matrix and affect tumor invasion and or metastasis. Cytoplasmic expression of p21 protein can be significantly correlated with invasion and metastasis. The correlation of these two proteins in thymoma has not been widely studied, hence we aimed to analyze the expression of MMP-2 and p21 and investigate their correlation with capsular invasion of thymoma AB. This cross-sectional study was performed on the 24 paraffin-embedded samples of thymomectomy during January 2013-Desember 2019 at Anatomical Pathology Laboratory of Dr. Soetomo General Hospital Surabaya. The samples were divided based on capsular invasion into 2 groups. Immunohistochemical staining was performed to detect expression of p21 and MMP-2. The correlation was statistically analyzed using Spearman test. There was no significant difference of MMP-2 expression between thymoma with capsular invasion and without capsular invasion (p=0.839), and also no significant difference of p21 expression between thymoma with capsular invasion and without capsular invasion (p=0.816). No correlation of MMP-2 and p21 expressions in thymoma AB was revealed (p=0.255). In thymoma AB, the expression of MMP-2 and p21 were not correlate with capsular invasion. These results may contribute to the development of thymoma research.

Keywords: Thymoma, MMP-2, p21, capsular invasion

Introduction

Thymoma is the most common neoplasm in the mediastinum, origin from the epithelial cells of the thymus gland, and the cause has not been determined until now[1]. The incidence of thymoma is approximately 1.3-3.2 cases per 1 million world population[2]. Their management strategies have not been standardized yet and because of the rarity case[1]. Thymoma is generally an indolent neoplasm, however, all subtypes of thymoma can appear at an advanced stage and show malignant behavior[3].

World Health Organization (WHO) 2015 divides thymoma into five subtypes which type A is reported to be about 4-7% of all types of thymoma, type AB 28-34%, type B1 9-20%, type B2 20-36%, and type B3 10-14%[4]. Tumor invasion and metastasis are also thought to be more related to prognosis than with tumor histology[5]. All thymoma subtypes have the potential to invade adjacent tissue with the rate of invasion increases according to tumor type in the order of types A, AB, B1, B2, B3. In thymoma A there were no invasion cases, in thymoma AB there were 5.9% cases, B1 18.5%, B2 20.5%, and B3 41.7% cases[6]. Many researches investigate about invasion in thymoma B2 and B3 but...
The most important part of the metastatic process is the degradation of the basement membrane and extracellular matrix. Basement membrane damage is the main predictor of tumor metastasis. Type IV collagen is the main component of the basement membrane. MMP-2 has a role in cell invasion because of its ability to degrade type IV collagen. Immunohistochemical examination of MMP-2 is also useful for showing a correlation with tumor stage, predicting aggressiveness (invasion) and the potential for malignancy of thymoma.

The p21 protein, also known as a cyclin-dependent kinase (CDK) 1 or protein-interacting 1 inhibitor, is an identified potent inhibitor of the cyclin/CDK complex. p21 in the cytoplasm has the function of promoting tumor proliferation and metastasis and in one study, it had a negative effect on thymoma survival rates, and was significantly correlated with WHO subtype, Masaoka stage and with thymoma invasion.

The correlation of these two proteins in thymoma, especially in thymoma AB has not been widely studied. This research aimed to analyze the expression of MMP-2 and p21 and investigate their correlation with capsular invasion of thymoma AB and get the prospect of providing prognosis of thymoma in general and thymoma AB specifically.

**Materials and Methods**

This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (2015/KEPK/VI/2020). This was analytic observational research with a cross-sectional approach that was performed on the 24 paraffin-embedded samples of thymectomy during January 2013-Desember 2019 at Anatomical Pathology Laboratory of Dr. Soetomo General Hospital Surabaya. The samples were grouped based on capsular invasion into two groups. Thymoma AB with capsular invasion were 13 samples and thymoma AB with no capsular invasion were 11 samples.

Immunohistochemistry staining was performed to detect expression of p21 and MMP-2. The tissues were cut into four mm sections, deparaffinized three times with xylol for five minutes each, and rehydrated through graded alcohol. Antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH 6.0) for ten minutes. The tissue sections were then incubated with monoclonal antibodies for MMP-2 (8B4: sc-13595; dilution 1:200; Santa Cruz Biotechnology) and p21 (0.N.488: sc-71811; dilution 1:200; Santa Cruz Biotechnology) overnight, followed by secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol.

Cytoplasmic staining for MMP-2 and p21 were evaluated in tumour cells. MMP-2 is considered positive if expressed in the cytoplasm >1% of tumor cells. p21 is considered positive if expressed in the cytoplasm >1% of tumour cells. All samples were evaluated by two pathologists in the blinded fashion. Any discordant was solved by interobserver agreement. The comparison of MMP-2 and p21 expression in thymoma AB with and without capsular invasion was tested using Mann-Whitney U test. The correlation was analyzed using Spearman test, a p-value of less than 0.05 was considered statistically significant.

**Results and Discussion**

The majority of patients in this research were aged 40-49 years (29.2%) and average age of all patients was 52.21 years. Clinicopathological characteristics of the patients are shown in Table 1. MMP-2 was expressed at cytoplasm (Figure 1). Mann-Whitney U test showed no difference in thymoma AB with and without capsular invasion (p = 0.839) (Table 2). p21 was expressed at cytoplasm (Figure 2). Mann Whitney U test showed no difference in thymoma AB with and without capsular invasion (p = 0.816) (Table 3). Spearman correlation test showed no correlation between MMP-2 and p21 in thymoma AB (r = 0.242, p = 0.255) (Table 4).
Table 1. Clinicopathological characteristics of the patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) #</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>40-49</td>
<td>7 (29.2)</td>
</tr>
<tr>
<td>50-59</td>
<td>6 (25)</td>
</tr>
<tr>
<td>60-69</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td>70-79</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (70.8)</td>
</tr>
<tr>
<td>Female male</td>
<td>7 (29.2)</td>
</tr>
</tbody>
</table>

Note: Mean age 52.21 years, range 34-79 years.

Table 2. Comparaison between MMP-2 expression with and without capsular invasion.

<table>
<thead>
<tr>
<th>MMP-2 EXPRESSION</th>
<th>P-VALUE#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>NO CAPSULAR INVASION</td>
<td>38.4</td>
</tr>
<tr>
<td>CAPSULAR INVASION</td>
<td>37.3</td>
</tr>
</tbody>
</table>

Mann-Whitney U test applied.

*p-value <0.05, considered as significant.

Table 3. Comparaison between p21 expression with and without capsular invasion.

<table>
<thead>
<tr>
<th>MMP-2 Expression</th>
<th>p-Value #</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>No Capsular Invasion</td>
<td>30.6</td>
</tr>
<tr>
<td>Capsular Invasion</td>
<td>30.4</td>
</tr>
</tbody>
</table>

Mann-Whitney U test applied.

*p-value <0.05, considered as significant.

Table 4. Correlation between MMP-2 and p21 expression in thymoma AB.

<table>
<thead>
<tr>
<th>p21 Expression</th>
<th>MMP-2 Expression</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs</td>
<td>0.242</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.25524</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>
The metastatic process is undoubtedly an important phase of neoplastic disease and develops when tumor cells acquire the specific ability to leave the primary tumor, invading the surrounding matrix\textsuperscript{[11]}. Matrix metalloproteinases (MMPs) and tissue metalloproteinase inhibitors (TIMPs) represented a critical role in tumor invasion and metastasis. In particular, gelatinase including gelatinase A (MMP2) can degrade the extracellular matrix (ECM) and basement membrane. Very few studies have been conducted on thymoma, and fewer papers have discussed the expression of the MMP molecule and its significance for predicting biological behavior in thymoma. It is known that the gelatinolytic activity of MMP2, known as gelatin zymography, is associated with thymoma invasion and is thought to have the potential for malignancy\textsuperscript{[12]}. Capsule invasion is a factor that can affect the prognosis of thymoma\textsuperscript{[13]}. The age of the patients observed in this study ranged from the age of 34-75 years (mean 52.21). Most belonged to a group of the 40-49 years (29.2%) and the majority occur in women (70.8%). This is consistent with a study stated that type A and AB patients were significantly older than patients B1-3 by a median of 60 versus 52\textsuperscript{[14]} and the majority patients are women with an overall mean age of 51.0 ± 14.3 years\textsuperscript{[15]}. The results of this study indicated that there was no significant difference between the expression of MMP-2 and capsular invasion in AB thymoma. According to another research conducted by Takahashi, the expression of MMP-2 showed varying results, namely in type A thymoma, expression 20%, type AB 8.3%, type B1 18.2%, type B2 27.3%, type B3 77.8%, type C 55.6%. Researchers also conducted research on TIMP-2 which the results were positive in 40%, 58%, 73%, 82%, 78%, and 67% respectively of types A, AB, B1, B2, B3, and C thymoma. The MMP-2 expression showed a weak correlation with TIMP-2 where the Spearman correlation coefficient is 0.362\textsuperscript{[5]}. While other studies mentioned, all thymomas A expressed MMP-2, 67% expressed TIMP-2, and about 66% of thymomas expressed both. About 60% of thymoma B1 and 25% of thymoma AB expressed TIMP-2 and MMP-2, and 50% both expressed strongly. Most of thymomas B2, B3, and C (83-100%) expressed MMP-2 and TIMP-2. The result of the study, in non-invasive (stage I) thymoma the positive staining rates for MMP-2 and TIMP-2 were very low (10% and 0%, respectively), and in invasive (stage II-IV) tumors, the positive staining rates for MMP-2 and TIMP-2 were very high (91% and 97%, respectively). There was a significant difference in the expression levels of MMP-2 and TIMP-2 between
non-invasive and invasive thymoma (Fisher exact test; P = 0.0001)\(^{[16]}\).

MMP activity is a balance between MMPs/TIMPs and is a determining factor for maintaining the stability and integrity of the extracellular matrix. The roles of MMPs in tumor metastasis were not relied on the exact concentration of MMPs in the local area, but on the ratio of MMPs/TIMPs\(^{[17]}\). TIMP is a specific regulator of MMP. Decreased TIMP production can also result in more effective enzyme activity and is potentially invasive. Overexpression TIMP in tumor cells can inhibited tumor invasion and metastasis\(^{[18]}\). MMP-2 and TIMP-2 imbalance is very important for tumor cells to have a strong invasive potential. Thus, exact concentration of MMPs in the local area has no significance and cannot reflect tumor potential. MMPs/TIMPs can act as a prognostic factor indicating invasiveness and metastasis\(^{[17]}\). The unexpressed MMP-2 in this study could be due to the inhibitory activity of TIMP-2, but the previous MMP-2/TIMP-2 ratio data were not known.

Regarding p21 expression, there was also no significant difference between the expression of p21 and capsular invasion in AB thymoma. This result is in accordance with study by Kuhn and Wistuba that stated the expression of p21 was minimally increased (16%) in the 31 examined thymomas\(^{[19]}\) and according to Omatsu, there was no cytoplasmic p21 expression in normal thymic epithelial, thymoma, and thymic carcinoma\(^{[20]}\). Also, p21 expression was detected in small amounts in thymoma and it has been reported that p21 expression is increased in thymic carcinoma than in thymoma B3. The combination of high p53, low p21 and p27 low expression has the potential to predict the biologically aggressive behavior of thymoma\(^{[21]}\) and it is found more frequently in invasive than non-invasive thymomas\(^{[22]}\).

The results of this study indicate that there is no relationship between MMP-2 and p21 expression in thymoma AB. A research suggests the expression of TIMP2 at p21 reduction, while TIMP2 is described as the component responsible for removing MMP. Taken together, reduction of p21 leads to attenuated cell motility and invasiveness of the different cell lines, possibly by ERK3 scattering which in turn results in decreased levels of the MMP2 and TIMP2 genes. In summary, the data suggest that p21 reduction has a negative impact on cell motility and invasion that is mediated, at least in part, by the ERK3 / MMP2 / TIMP2 pathway\(^{[23]}\).

**Conclusion**

In thymoma AB, the expression of MMP-2 and p21 were not correlate with capsular invasion. These findings can contribute to an improvement of thymoma research.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Source of Funding :** This study supported by the Ministry of Education and Culture of the Republic of Indonesia.

**Acknowledgements:** We thank Arif Nur Muhammad Ansori for editing the manuscript. We additionally to thank Dr. Budi Utomo, Department of Public Health and Preventive Medicine, Universitas Airlangga for statistical analysis.

**Ethical Approval:** This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (2015/KEPK/VI/2020).

**References**


Effect of Graded Theraband Exercise on Myofascial Dysfunctions in Breast Cancer Survivors

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Abstract

Background: Cancer is the leading cause of morbidity and mortality. Breast cancer is the most common form of cancer among women. Many breast cancer surgeries lead to dissection of muscles and soft tissue structures. After the breast cancer treatment, many complications are seen in breast cancer survivors. In that myofascial dysfunctions are common. These dysfunctions are one of the common sources of pain and discomfort. Not only pain but this dysfunction also leads to decreased range of motion, decreased strength, and altered posture. Because of dysfunctions, an upper body function decreases and affects the activity of daily living. Graded theraband exercises are effective in improving muscle strength and shoulder range of motion in breast cancer survivors. And also helps to enhance functional capacity. Early rehabilitation can be implanted to reduce pain, improve strength and flexibility which will be helpful in minimize restrictions in daily living activities and improve quality of life. This effectiveness study will help to enlighten the status of myofascial dysfunctions in breast cancer survivors. The objectives of the study were as follows: To find out the effect of graded theraband exercise on myofascial dysfunctions in breast cancer survivors.

Methods: A total 40 females who underwent breast cancer surgeries along with chemotherapy or radiotherapy or hormone therapy, were included based on inclusion criteria. All the females received theraband exercises along with medications for five days per week and pre and post assessment was done with manual muscle testing (MMT), shoulder range of motion (ROM), and Shoulder Pain and Disability Index (SPADI) questionnaire.

Result: The result concluded that strengthening with thera-band showed better effect in the breast cancer survivors. Strengthening exercise program with resistance band showed significant improvement in the shoulder strength, ROM and activities of daily life.

Keywords: SPADI, MMT, Thera-band, myofascial dysfunctions, Breast cancer survivors (BCS)

Introduction

Breast cancer refers to cancers originating from breast tissue, most commonly from the inner lining of milk ducts or lobules that supply ducts with milk¹. Prevalence of breast cancer in India is 25.8 per 100000². When cancer cells metastasize to nearby tissues or to distant areas of the body it is known as malignant tumor³. When cancer cell grows but does not spread it is known as benign tumor. There are many risk factors such as sex, aging, estrogen, family history, gene, unhealthy lifestyle which increases the risk of developing breast cancer⁴. Since from few years, awareness is increased about breast cancer. Many surgeries are performed to remove the lump. Recently more selective and less invasive methods of treatments are available⁵. Breast cancer treatment includes surgery, chemotherapy,
radiation, hormone therapy, it can be used alone or with combination. This increases life expectancy for women in breast cancer and they have given special importance to improve quality of life. After the surgeries many complications are seen in that pain, reduced total loss of movements, muscle strength, reduce flexibility, postural changes, scarring complications, sensitivity alterations. Many breast cancer survivors reported significant limitations in upper body strength which is important for daily life activities such as pushing, lifting and reaching to an object especially problematic for the Indian women. Patients having difficulty to completing task. Also it affects on patients quality of life. Breast cancer survivors have muscle weakness of upper limb. Chemotherapy shows a loss of muscle mass. The muscle weakness can also progress and lead to loss hand function. Mainly in the breast cancer management muscles of shoulder complex are involved which causes difficulty in moving hand.in breast cancer management pectoral muscles, rhomboid, serratus ant, trapezius are important which causes difficulty in flexion, abduction, internal rotation of the shoulder joint. Graded theraband exercises have shown interesting effects in reducing fatigue levels, improve functional capacity, and muscle strength and inducing positive changes in body composition which shows direct effect on patients quality of life. This graded theraband exercises is useful in improving functional capacity, muscle strength and hand mobility. Thera-bands are cost effective, easy to handle, portable, and versatile. Theraband exercises are very easy and simple, hence patient can do easily at home also.

**Materials and Method**

It was an interventional study which was carried out in Krishna hospital, karad. The study duration was the 6 months. The study was conducted in Krishna Hospital, Karad. An informed consent was taken from the subjects who were included in the study. Total numbers of subjects included in the study were 40, this sample size was calculated using formula \( n = \frac{4pq}{L^2} \). Subjects were taken by using simple random sampling techniques. The duration of the study was 6 months, and the treatment was given for 30 minutes per day and 5 days / week. All the subjects were included in this study based on inclusion criteria. An inclusion criterion includes breast cancer survivors, stage I and stage II A. Histological confirmed breast cancer survivors with no evidence of recurrent or progressive disease since last six months. Completed surgery, radiotherapy and or chemotherapy. Females with age between 40 to 55 years. Breast cancer survivors receiving hormone therapy. An exclusion criteria includes breast cancer survivors with known case of fracture dislocation, stroke, peripheral neuropathy, skin diseases. Infections in the axillary area. Materials used in this study are Thera-band, SPADI Questionnaire and Goniometer.

<table>
<thead>
<tr>
<th>Thera-band colour</th>
<th>Resistance level</th>
<th>Workout level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>Thin</td>
<td>Beginner</td>
</tr>
<tr>
<td>Red</td>
<td>Medium</td>
<td>Beginner/intermediate</td>
</tr>
<tr>
<td>Green</td>
<td>Heavy</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

All the exercises are started with 2-3 sets of 10-15 repetitions. Gradual progression to next colour when breast cancer survivors is able to easily complete 3 sets of 10-15 repetitions.

1. The Shoulder Pain and Disability Index (SPADI) is the self-administered questionnaire that consists of two dimensions, one for pain and the other for functional activities. The pain dimension consists of five questions related to severity of pain. Functional activities are assessed with eight questions design to measure the difficulty an individual has with various activities of daily living that require upper extremity use.
2. Manual Muscle Testing (MMT): It is the method of diagnostic evaluation used by physiotherapist. It is the important part of physical therapy examination which is useful in differential diagnosis and treatment of musculoskeletal and neuromuscular disorders. It is graded from 0-5 which is determined by the patients capability to move tested body part depending upon muscle contractility, gravity assisting and antigravity positions.

Procedure

The study was conducted to find out the effect of graded theraband exercises on myofascial dysfunctions in breast cancer survivors. After getting ethical approval from the institutional ethical committee of Krishna institute of medical sciences “Deemed To Be University”, Karad. The total 40 subjects were selected as per inclusion and exclusion criteria. All the subjects were assessed for disabilities of the shoulder function and strength, mobility of the shoulder complex before intervening with the treatment. Subjects were explained about the procedure and importance of the study. The subjects received exercises of shoulder flexion, extension, abduction, adduction, internal rotation, external rotation by using theraband. All the exercise started with 2-3sets of 10-15 repetitions. The exercise started with the yellow colour theraband and progressed to red and then green colour. Progression to next colour considered when individual was able to easily complete 3 sets of 10-15 repetitions. The study was done 5times per week for 8 week. After the 8 weeks the post treatment assessment for shoulder range of motion, disability and strength by using SPADI Questionnaire, goniometer, MMT. After that the subjects taken for statistical analysis and the result of the study was done on the basis of comparing pre and post-test assessment. The study resulted by statistical analysis of the entire outcome measures.

Statistical Analysis

Statistical analysis was done manually and by using instat software to verify the result. The statistical analysis was done by using paired T-test. P and T test was calculated by using instat software. Effect of graded theraband exercises on myofascial dysfunctions in breast cancer survivors was analysed.

Result

Data Presentation

Table no.1 Sociodemographic data

<table>
<thead>
<tr>
<th>AGE</th>
<th>PRESENT</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above50</td>
<td>23</td>
<td>57.5</td>
</tr>
<tr>
<td>Below50</td>
<td>17</td>
<td>42.5</td>
</tr>
<tr>
<td>PAST HISTORY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRESENT</td>
<td>23</td>
<td>57.5</td>
</tr>
<tr>
<td>TREATMENT HISTORY</td>
<td>PRESENT</td>
<td>PERCENTAGE</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Hormonal therapy</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Surgery</td>
<td>21</td>
<td>52.5</td>
</tr>
<tr>
<td>WORKING STATUS</td>
<td>PRESENT</td>
<td>PERCENTAGE</td>
</tr>
<tr>
<td>Workers</td>
<td>19</td>
<td>47.5</td>
</tr>
<tr>
<td>Non-workers</td>
<td>21</td>
<td>52.5</td>
</tr>
<tr>
<td>HABBITS</td>
<td>PRESENT</td>
<td>PERCENTAGE</td>
</tr>
</tbody>
</table>
**Interpretation:** Above table represents sociodemographic data that includes age, treatment history, habits, working status, obesity and past history; which gives information about patients history.

**Table no. 2: Shoulder Manual Muscle Testing.**

<table>
<thead>
<tr>
<th></th>
<th>Flexion</th>
<th>Extension</th>
<th>Abduction</th>
<th>Adduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>2.25</td>
</tr>
<tr>
<td>Post</td>
<td>2.9</td>
<td>2.9</td>
<td>3.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

**Interpretation:** Above table shows pre and post comparison within the group. Post treatment showed significant improvement seen in strength of shoulder according to prevalence rate.

**Table no. 3: Shoulder Range of Motion.**

<table>
<thead>
<tr>
<th></th>
<th>Flexion</th>
<th>Extension</th>
<th>Abduction</th>
<th>Adduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>99.3</td>
<td>19.6</td>
<td>99.4</td>
<td>24.3</td>
</tr>
<tr>
<td>Post</td>
<td>140.8</td>
<td>25.8</td>
<td>113.1</td>
<td>38.3</td>
</tr>
</tbody>
</table>

**Interpretation:** The above tables show pre and post comparison of the treatment. Post treatment showed significant improvement in shoulder range of motion.

**Table no. 4: The Shoulder Arm Disability Index (SPADI)**

<table>
<thead>
<tr>
<th></th>
<th>Pre test</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>81</td>
<td>72.9</td>
</tr>
</tbody>
</table>

**Interpretation:** Above table shows pre and post comparison within the group. Post test showed significant improvement seen in shoulder mobility by using SPADI questionnaire.
Discussion

Many studies are undertaken to find out the prevalence rate of myofascial dysfunctions but there was not awareness about the rehabilitation programme. This dysfunction have direct relation with the QOL of breast cancer survivors that’s why it was necessary to put some light on rehabilitation programme. It will help to improve QOL and functional mobility in breast cancer survivors. Knowing about the importance of the research, this topic has been approved by institutional ethical committee and there after research began. Sample size included in this study was 40 females which was selected by simple random sampling techniques who are undergone breast cancer management. After that the protocol is given to each patient and explain about all the information and importance of the study. This study is on “effect of graded thera-band exercises on myofascial dysfunctions in breast cancer survivors” was conducted to find out the effect of strengthening with thera-band and find the effect on muscles strength and arm mobility. Breast cancer survivors have shown interesting effect to decrease fatigue level, improve functional capacity and muscle strength. Graded thera-band exercises showed improvement in SPADI scores with prevalence rate <0.0001 and showed improvement in shoulder movements. The result showing that strengthening with thera-band showed better effect compared to previous status of breast cancer survivors. Also graded thera-band exercises helps to improve patients quality of life. Thera-band strengthening exercises have been shown to be practicable alternative to weight cuffs and other strength training methods. Thera-bands are easily available in hospitals and physiotherapy clinics. This thera-band easy to use, breast cancer survivors can also do all the exercises at home also. When comparing this research with previous studies, Ausanee Wanchai (2018) concluded that resistance exercises is beneficial for effective lymphedema management. Where in our study exercise programme is focused on myofascial dysfunctions related to upper body which shows better effect and progression in health status of breast cancer survivors. Roger T. Anderson showed that many of the studies used manual resistance exercises. In which the resistance is applied by the therapist, where in our study resistance force is applied by the thera-bands which are easy to handle and more convenient. Cornie et al. concluded that progressive resistance exercises significantly improved muscle strength, muscle endurance and quality of life in women with breast cancer related lymphedema. In present study thera-band exercises started earlier in breast cancer survivors. When the body system are started exposed to a greater than usual but proper level of resistance, they react with a number of acute physiological response and later adapt to the newly imposed physical demands. In thera-band exercises, the speedy get to in tension producing capacity of skeletal muscles is largely attributed to neural responses not adaptive muscle changes itself. All of this response might have contributed in improvement in SPADI questionnaire. Comparably high levels of muscle activation during exercises are achieved resistance training with elastic products than weights and it is feasible alternative to training with machines and heavy weights. Also, resistance training with thera-band is subjective force as the subject might stretch minimally or as tolerated the initial few days and as neural and muscle adaptation occurs may more stretch more to experience more resistance. Which will eventually add to increase in flexibility and strength, which isn’t the case in traditional weights as the resistance cannot be altered. Although it is known that regular exercises and physical activity can improve health related status of the breast cancer survivors. There is a critical need for developing correct treatment and early rehabilitation protocols related to myofascial dysfunctions which causes negative effect on patients health status. To improve activity of daily and quality of life graded theraband exercises are needed.

Conclusion

The present study concluded that the thera-band exercises improved better strength than the previous status of breast cancer survivors. All the activities of daily living also improved and that is confirmed again with the SPADI questionnaire.

Conflicts of Interest: The authors declare that there are no conflicts of interest concerning the content of the present study.

Funding Source: This study was funded by Krishna Institute of Medical Sciences “Deemed to Be University Karad, Maharashatra.

Ethical Approval: From institutional ethical committee of Krishna Institute of Medical Sciences
Deemed to Be University Karad, Maharashtra.

**Abbreviations:**

MMT: Manual Muscle Testing

ROM: Range of Motion

SPADI: Shoulder Pain and Disability Index

**Acknowledgement:** We acknowledge the guidance and constant support of Dean, Faculty of Physiotherapy, KIMSDU, Karad; Dr. Mandar Malawade and Dr. Kakade SV for help in statistical analysis.

**References**


Morphometric study of Macewan’s Triangle in Relation to Depth of the Sigmoid Sinus Plate

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Abstract

Background: The suprameatal triangle is used for approaching the tympanic cavity and also it is an important landmark for otologic surgeons during mastoidectomy.

Aim: To determine the depth of sigmoid sinus plate over the Macewan’s triangle by morphometry of various anatomical landmarks.

Materials & Methods: The study was carried out in the Department of Anatomy, Penang International Dental College & VMKV Medical College, Salem on 30 dry human adult skulls. Five landmarks (D1, D2, D3, D4, D5 and D6) were taken on the left and right sides of the skulls and the imaginary lines were constructed and measured by Vernier caliper between the landmarks.

Results: The measurement of D2 showed statistically significant differences. The correlations of D1, D3, D4, D5 and D6 on both sides do not show any significant differences. The linear correlation equations were derived using the measurements for predicting D6.

Conclusion: The depth of sigmoid sinus plate (D6) can be assessed using D1, D2, D3, D4, D5 on lateral view of head & neck X-rays which can be used by Otologist during mastoidectomy to avoid severe bleeding complications from sigmois sinus.

Keywords - Henle’s spine, Suprameatal triangle, Mastoidectomy, Sigmoid sinus,

Introduction

The Macewan’s triangle or suprameatal triangle is an important surgical landmark for mastoidectomy. It is used by surgeons as an anatomical landmark to locate the mastoid antrum of the mastoid process. Suprameatal triangle is present above external acoustic meatus of the temporal bone. The triangle is related to the Henle’s spine or suprameatal spine. The suprameatal spine is crest shape in female and suprameatal depression is deeper in males than in females. The mastoid antrum of the mastoid process is located 15 mm deeper to the suprameatal triangle of the skull. The suprameatal triangle is bounded anteriorly by the post-superior border of the external auditory canal, superiorly by the supramastoid crest and posteriorly by a vertical line drawn tangential to the posterior margin of the external canal.

The supramastoid crest corresponds to the floor of middle cranial fossa. The posterosuperior margin of the...
external acoustic meatus, lies over the descending part of the facial nerve canal. The suprameatal approach can avoid the process of mastoidectomy and posterior tympanotomy. The suprameatal approach is done by entering through retroauricular tympanotomy flap, by introducing an electrode through the tunnel drilled in the suprameatal triangle above the Henle’s spine. Thus the approach is of great importance to otologic surgeons during mastoidectomy.

The sigmoid venous sinus commences immediately below the temporal bone on both sides of the head, following a complex course by passing through sigmoid sulcus and enters the jugular foramen and continues down as the internal jugular vein. In mastoid clearance two main surgical procedures were followed that includes canal wall up and canal wall down. The sigmoid sinus acts as an anatomical marker during mastoidectomy. The anatomy of sigmoid sinus and its extent along with the depth of it from suprameatal triangle will help to prevent unwanted bleeding during surgery.

**Aim:** To determine the depth of sigmoid sinus plate over the Macewen’s triangle by morphometry of various anatomical landmarks.

**Objectives**

1. To correlate the surgical landmarks on the lateral surface of the mastoid bone to the sigmoid sinus plate depth.

2. To trace out the depth between the Macewen’s triangle and the sigmoid sinus plate using various surgical landmarks.

**Materials and Methods**

The study was carried out in the Department of Anatomy, Penang International Dental College & VMKV Medical College, Salem on 30 dry human adult skulls. The study was approved by IEC of VMKV Medical College (Reference - VMKVMC/IEC/18/52). Five landmarks were used on the left and right sides of the skulls which includes the tip of the mastoid process, the asterion, and the midpoint of the suprameatal triangle, suprameatal spine/Henle’s spine and porion. Five imaginary lines, D1: Distance between asterion and tip of mastoid process, D2: Distance between asterion and midpoint of Macewen’s Triangle, D3: Distance between tip of mastoid to midpoint of Macewen’s Triangle, D4: Distance between Henle’s spine and midpoint of Macewen’s Triangle, D5: Distance between porion and midpoint of Macewen’s Triangle D6: Depth of Macewen’s Triangle. To measure the depth of sigmoid sinus plate from the surface of suprameatal triangle (D6), the skull was oriented in the anatomical position (Frankfurt plane) and the drilling was done using electrical driller between surface of suprameatal triangle and the sigmoid sinus plate towards the direction of maxillary process of temporal bone along its upper border. Skulls without any damage of Macewen’s triangle, prominent suprameatal spine/ Henle’s spine were included for the study.

**Results**

D1, D2, D3, D4, D5 and D6 of adult skulls were measured on both right and left side and the average was calculated among the sides and tabulated as below.
(Table1). The average of D1 (5.14±0.45 cm), D2 (3.87±0.18 cm), D3 (3.35±0.38 cm), D4 (3.27±0.14 cm), D5 (1.22±0.19 cm) and D6 (0.93±0.043 cm) of all 30 skulls on both sides (Table1).

Table 1: Variants of D1, D2, D3, D4, D5 and D6 of adult skulls

<table>
<thead>
<tr>
<th>Variants</th>
<th>Right (cm)</th>
<th>Left (cm)</th>
<th>Average(cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>4.97 ± 0.58</td>
<td>5.30 ± 0.14</td>
<td>5.14±0.45</td>
</tr>
<tr>
<td>D2</td>
<td>3.99 ± 0.17</td>
<td>3.76 ± 0.11</td>
<td>3.87±0.18</td>
</tr>
<tr>
<td>D3</td>
<td>3.69 ± 0.14</td>
<td>3.0 ± 0.16</td>
<td>3.35±0.38</td>
</tr>
<tr>
<td>D4</td>
<td>3.29±0.15</td>
<td>3.26±0.12</td>
<td>3.27±0.14</td>
</tr>
<tr>
<td>D5</td>
<td>1.19 ± 0.17</td>
<td>1.26 ± 0.19</td>
<td>1.22±0.19</td>
</tr>
<tr>
<td>D6</td>
<td>0.93 ± 0.17</td>
<td>0.94 ± 0.05</td>
<td>0.93±0.043</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD, n = 30 with 30 on right side & 30 on left side of skull. **D1**: Distance between asterion and tip of mastoid process, **D2**: Distance between asterion and midpoint of Macewen’s Triangle, **D3**: Distance between tip of mastoid to midpoint of Macewen’s Triangle, **D4**: Distance between Henle’s spine and midpoint of Macewen’s Triangle, **D5**: Distance between porion and midpoint of Macewen’s Triangle. **D6**: Depth of Macewen’s Triangle.

The variants of both right and left side measurements suggested the linear correlation (R value) between variables which was measured by Pearson correlation coefficient. D2 was found to be statistically significant (0.002) when compared to other variants (Table 2) (Figure 2)

Table 2: Statistical Correlations and the probability (p values) of variants

<table>
<thead>
<tr>
<th>Variants (Right &amp; Left)</th>
<th>Correlation (R value)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>0.0918</td>
<td>0.529#</td>
</tr>
<tr>
<td>D2</td>
<td>0.42598</td>
<td>0.002*</td>
</tr>
<tr>
<td>D3</td>
<td>0.23138</td>
<td>0.106#</td>
</tr>
<tr>
<td>D4</td>
<td>0.06815</td>
<td>0.638#</td>
</tr>
<tr>
<td>D5</td>
<td>0.12874</td>
<td>0.373#</td>
</tr>
<tr>
<td>D6</td>
<td>0.00868</td>
<td>0.952#</td>
</tr>
</tbody>
</table>

n = 30 with 30 on right side & 30 on left side of skull , # - non significant, * - significant, P value *P<0.05, Statistical analysis – Pearson correlation coefficient. **D1**: Distance between asterion and tip of mastoid process, **D2**: Distance between asterion and midpoint of Macewen’s Triangle, **D3**: Distance between tip of mastoid to midpoint of Macewen’s Triangle, **D4**: Distance between Henle’s spine and midpoint of Macewen’s Triangle, **D5**: Distance between porion and midpoint of Macewen’s Triangle. **D6**: Depth of Macewen’s Triangle.
The variants of both right and left side measurements suggested the linear regression ($R^2$ value) between variables. All variants were found to be statistically significant when compared (Table 3).

### Table 3: Linear regression and (D6) equation derived

<table>
<thead>
<tr>
<th>Linear regression (R2 Value)</th>
<th>Equation Derived</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.004*</td>
<td>D6 = 0.0056 D1 + 0.9056</td>
</tr>
<tr>
<td>0.012*</td>
<td>D6 = -0.0257 D2 + 1.0341</td>
</tr>
<tr>
<td>0.010*</td>
<td>D6 = -0.0115 D3 + 0.9731</td>
</tr>
<tr>
<td>0.017*</td>
<td>D6 = -0.0408 D4 + 1.0681</td>
</tr>
<tr>
<td>0.001*</td>
<td>D6 = -0.002 D5 + 0.938</td>
</tr>
</tbody>
</table>

n = 30 with 30 on right side & 30 on left side of skull, # - non significant, * - significant, P value *P<0.05, Statistical analysis – Linear regression

![Figure 2: Linear regression and (D6) equation derivation](image-url)
**D1**: Distance between asterion and tip of mastoid process, **D2**: Distance between asterion and midpoint of Macewen’s Triangle, **D3**: Distance between tip of mastoid to midpoint of Macewen’s Triangle, **D4**: Distance between Henle’s spine and midpoint of Macewen’s Triangle, **D5**: Distance between porion and midpoint of Macewen’s Triangle, **D6**: Depth of Macewen’s Triangle.

**Discussion**

The morphology and anatomy of the suprameatal triangle has more surgical importance. The knowledge is very much essential for otologic surgeons to approach the mastoid antrum. Sigmoid sinus is a dural venous sinus draining the brain structures. Emissary vein drains into the sigmoid sinus through the posterior condylar canal. The sigmoid sinus is related to the occipital emissary foramen which transmits a vein that connects sigmoid sinus with the suboccipital venous plexus of veins. During mastoidectomy, there is a complication of perforating the sigmoid sinus. The perforation of sigmoid venous sinus during mastoidectomy can be prevented by calculating the depth of the sinus plate preoperatively using the equation derived. It is very important to know the exact depth and site of sigmoid sinus. In radical mastoidectomy chances are more for damage of the sinus plate accidentally which can result in severe venous bleeding. If the depth (D6) can be calculated from D1, D2, D3 and D5 on the lateral view of plain X-rays, the dangerous complication of severe venous bleeding can be avoided. The statistical analysis of the variants suggested that their exists correlation between the depth of the sinus plate (D6) with other five parameters namely D1, D2, D3, D4 and D5.

The depth of the sigmoid sinus plate can be calculated using various measurements of D1 to D5 using X-rays with Macewen’s triangle as a surgical landmark. Many morphometric measurements were performed and reported between various surgical landmarks for the mastoideotomy. The suprameatal approach was proved to be safer technique as it does not endanger either facial nerve or the chorda tympani nerve.

Mohammad et al., 2011 were using D1 and D2 to estimate D3 (depth of sigmoid sinus plate), in their study and stated a hypothesis that measuring the first two lines D1 and D2 might be an indicator to the third line D3. Earlier studies were done only on the topographic anatomy and surgical importance of the suprernal triangle but no further studies had mentioned the use of various landmarks of Macewen’s triangle to know the depth of sigmoid sinus plate. The equation derived are as follows, D6 = 0.0056 D1 + 0.9056, D6 = -0.0257 D2 + 1.0341, D6 = -0.0115 D3 + 0.9731, D6 = -0.0408 D4 + 1.0681, D6 = -0.002 D5 + 0.938 (Table 3).

In the present study D2 (p value - 0.002) showed statistical significant differences (Table 2). By finding D1, D2, D3 and D5 radiologically, the approximate depth of sigmoid plate (D6) value can be calculated. D4 cannot be used as a reliable landmark as the spine may be absent or not able to trace out in X-ray image.

**Conclusion**

The depth of sigmoid sinus plate (D6) can be calculated using the measurements of D1, D2, D3, D4, D5 on lateral view of head & neck X-rays which can be used by Otologist during mastoidectomy to avoid severe bleeding complications from sigmois sinus. The pre-operative measurement can be taken well in advance using the radiographic images like X-rays and the surgery can be planned accordingly.

**Ethical Committee Approval:**

The study was approved by IEC of VMKV Medical College (Reference - VMKVMC/IEC/18/52).

**Conflict of Interest:** Nil

**Source of Funding:** Self funding

**References**


A Comparative study on Prevalence of Diastasis Recti in Primipara and Multipara Undergone Full Term Normal Delivery - A Research Protocol

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Abstract

Background: Diastasis Recti is very common in women after pregnancy. Diastasis recti among women should be treated as soon as possible as it may cause various musculoskeletal dysfunctions. A digital caliper is a tool for assessing diastasis recti in postpartum women. It is a simple, fast, and reliable assessment tool for assessing diastasis recti. Objective: The objective of the study is to find the prevalence of Diastasis Recti in Primipara and Multipara undergone full-term normal delivery and to compare the prevalence of diastasis recti in the both of the groups Method: This study will be carried out in Physiotherapy OPD, Ravi Nair Physiotherapy College and AVBRH, Sawangi (Meghe), Wardha. Diastasis recti will be evaluated in full-term normal delivery females. Diastasis recti will be compared in primipara and multipara using a digital caliper. Results: The result of the study will be estimated by the statistical analysis of the data and will be discussed after the study is completed. Conclusion: After going through various studies, many studies have indicated that the prevalence of diastasis recti abdominis is commonly seen in multipara undergone cesarean section delivery. Very few studies indicate the prevalence of diastasis recti abdominis in primipara and also women underwent full-term normal delivery. Therefore, the current study is carried outreach a conclusion to find whether there is any discrepancy of the prevalence of diastasis recti abdominis between primipara and multipara in females undergone full-term normal delivery.

Keywords: Diastasis recti, Primipara, Multipara, Full term normal delivery (FTND)

Introduction

Diastasis of recti is one of the commonest consequences of pregnancy. Diastasis recti is caused by the separation of the two bellies the rectus abdominis along the linea alba and widening of the linea alba. There are certain factors which cause diastasis recti which is mechanical effect of pregnancy on the abdominal musculature or hormonal factors. Weakening of the linea alba results because of the softening of the connective tissue as there is increased level of relaxin, progesterone, and estrogen during pregnancy. Diastasis recti abdominis also causes low back pain which can be treated surgically. Exercises are recommended to the pregnant women which has many benefits which includes maintaining strength, muscle tone, and endurance. Exercises also helps to improve well-being of the patient, reduces labor pain and also low back pain. Multiparous women have an increased risk of development of diastasis recti because there is repeated and prolonged stretch on abdominal musculature. Pregnant women have weaker abdominals than non-pregnant women. Exercises are helpful in improving the strength and tone of the musculature. It helps to decrease and reduce the size of diastasis recti abdominis. There is weakening of the anterior abdominal wall because of strain placed by the enlarging uterus which results in diastasis recti. The rectus abdominis muscle becomes stretched and elongated around the enlarging uterus as the pregnancy advances. The main role of the abdominal

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Musculature is trunk control. The functions which result in diminished trunk stability, abnormal posture. (1) There are various methods for the assessment of diastasis recti abdominis which include the finger-width method, ultrasonography, magnetic resonance imaging (MRI), Computed tomography (CT) scan, calipers, and tape measurement. Diastasis recti abdominis can be treated conservatively focusing on postnatal exercises which limits the progression of diastasis recti abdominis, increases well-being of the patient, promotes weight loss, and also improves the cardiovascular endurance of the patient. Surgical management is needed which is (abdominoplasty) if a woman is unable to perform optimal functions like transfer of weight through the pelvic girdle. Parity increases the prevalence of diastasis recti. Diastasis recti abdominis is found both in primipara and multipara but prevalence of diastasis recti below the umbilicus is more in multiparous women and diastasis recti abdominis at the level of umbilicus is same in primipara and multipara. Diastasis recti is found more in multipara than in primipara. There is a link of diastasis recti abdominis with other conditions like urinary incontinence, fecal incontinence, uterus prolapse, myofascial pelvic pain, rectal prolapse, bladder prolapse. Diastasis recti abdominis also has an association with lumbopelvic pain. Tape measurement and finger-width method are used to measure diastasis recti abdominis because of its cost-effectiveness and accessibility. Digital caliper is used in the assessment of diastasis recti which shows a widening of inter rectal distance more than 2.5 cm at one or more assessment points (2) Diastasis recti is seen comparatively more in multipara than in primipara (3) Diastasis recti abdominis is found more in obese, multiparous, and multiple pregnancies. Including multiparous and multiple births diastasis recti abdominis is also found in women who have narrow pelvis because in pregnancy location of the baby will be more anteriorly. Diastasis recti abdominis can be umbilicus or can be extended above and below the umbilicus or even include the linea alba (4) There is little understanding of diastasis rectus abdominis in society. Majority of women are unaware of diastasis recti abdominis and do not know if they even have this problem. These women also are not aware of the possible exercises which can be performed. The exercises focus on the abdominal muscles and the exercises could be performed during pregnancy. Pregnancy without complications may be an encouragement for women who had a sedentary lifestyle before pregnancy. Women with sedentary lifestyle should be encouraged to add appropriate aerobic exercises and strengthening workout to their routine. Women who are physically active even before pregnancy should continue their lifestyle which includes working out daily, performing aerobic exercises, strengthening exercises. Exercises help in improving physical pain response, it also enhances proper body posture, if exercises are properly chosen then it also reduces pain in the lumbar spine area. This helps after delivery and also taking care of the newborn. Every other woman may have diastasis rectus abdominis after pregnancy but appropriate treatment may reduce the inter recti distance. It is necessary to raise awareness among women about the nature of diastasis rectus abdominis, its predisposing factors, its implications, and physiotherapy treatment. There are two groups of women with diastasis rectus abdominis after pregnancy. The first group in which women are able to regain proper load transfer through the abdominal wall with diastasis recti or without diastasis recti. In the second group of women diastasis, rectus abdominis is larger than the normal ones but they are unable to transfer load through the abdominal wall. In this group women are not able to perform activities like squatting, standing on one leg, changing positions for example sitting to standing, climbing stairs, walking. In this case, a woman requires abdominal surgery which is abdominoplasty which is done after delivery because there is long term damage to the abdominal muscles and the central fascia structures which means that the proper functioning cannot be obtained. Strengthening of the abdominals before pregnancy often decreases the risk of diastasis rectus abdominis, and if it happens then the size of the diastasis is small. It is also said that performing strengthening exercises of abdominals before pregnancy and continuing the exercise in the period of pregnancy can reduce the chances of having a Caesarean section delivery and also influence more effective delivery. (5) Most women experience an increase in inter-recti distance in the abdominal muscle during pregnancy or after pregnancy which occurs because of stretch in the linea alba or due to thinning of the linea alba. Diastasis of the abdominal muscle is considered to be pathologically positive if there is >2.7 cm widening of diastasis recti above the umbilical level. Diastasis of rectus abdominis occurs in
the second trimester of pregnancy and most frequently diastasis is seen in the third trimester of pregnancy. Few studies have been conducted which shows that there is an increase in the inter-recti distance at the 14th week of pregnancy and it continues to increase until delivery. Recovery of the diastasis of the rectus abdominis occurs between 1 day and 8 weeks after delivery. Diastasis of the rectus abdominis ranges from 66% to 100% in the third trimester of the pregnancy and then 53% after delivery. Diastasis recti can be treated surgically which can reduce the effect of the diastasis recti such as back pain. If regular exercises are done before and during the antenatal period such as the strengthening of the core muscles, then there is a reduced risk of developing diastasis recti and also if diastasis recti occur then to reduce the size of diastasis recti. Abdominal exercises are prescribed to the women after delivery who have diastasis of rectus abdominis. Other non-surgical interventions to treat diastasis recti include back care and postural education, aerobic exercises, and external support such as corsets and Tubigrip. Tone, control, and strength of the abdominal muscle can be maintained if the exercises are done, as exercise reduces the stress on the linea alba. Women who exercise regularly in their pregnancy have a less and reduced risk of developing diastasis recti than the women who have a sedentary lifestyle and who do not exercise regularly. Exercises performed in the antenatal period have faster recovery of diastasis recti abdominis. Ultrasound and digital calipers are used to measure the diastasis recti abdominal width and these are the reliable ones. Palpation and finger-width methods are also used to measure the diastasis recti abdominal width but they are not much reliable and not considered to be valid to measure the exact inter-recti distance. (6)

RATIONALE:

Various studies have been carried out to find prevalence of diastasis recti in multipara but very few studies indicate the presence of diastasis recti in primipara and in females with full-term normal delivery. Therefore, current study is carried out to find the prevalence of diastasis recti in females with full-term normal delivery and also to compare it among primipara and multipara.

Objectives

1. To Evaluate Diastasis recti in Full Term Normal Delivery Females
2. To Compare Diastasis Recti in Primipara and Multipara

Methodology

The study will be conducted in OPD of Community Health Science in Ravi Nair Physiotherapy College, Sawangi, (Meghe) Wardha. Prerequisite permission and IEC approval will be taken subjects will be consented and selected as per the inclusion criteria those who are willing to participate will be selected as samples. Assessment of diastasis recti will be done using dial caliper. Outcome measure calibration will be done as per width of diastasis recti in inches. Reading will be spread on a master sheet. Data will be analyzed statistically.

OUTCOME MEASURES: Width of Diastasis Recti using a dial caliper

METHODS:

Study design: Observational study
Study setting: Ravi Nair Physiotherapy College and AVBRH hospital Sawangi (M), Wardha

PARTICIPANTS:

Inclusion criteria:
1. Primipara with FTND
2. Multipara with FTND
3. Up to 6 months postpartum
4. Age 20 to 35 years

Exclusion criteria:
1. Female undergone C – section
2. Female with a previous history of abdominal surgeries
3. Female with BMI > 40 kg/m2

VARIABLE:

1. Width of diastasis recti
DATA SOURCE/MEASUREMENT:

For Diastasis recti – width of the gap between the two bellies of diastasis recti will be measured using a digital caliper

Bias: Age and other Anthropometric factors between the two groups will be matched and subjection not fulfilling the selection criteria will be excluded to prevent bias

Study size: 175

Statistical method: Simple random sampling

Result: Upon completion of the study results, statistical analysis will be estimated.

Discussion

The current study will be carried out to compare prevalence of diastasis recti in primipara and multipara. Various studies have reported a prevalence of diastasis recti in females undergone c- section whether may be primipara or multipara due to separation of rectus abdominis muscle during surgery. However, diastasis also occur due separation of two bellies of recti muscle to accommodate the growing size of uterus but after delivery in postpartum period when the structures of abdomen and pelvic come to their normal position eventually the gap is reduced but diastasis may still persist may show different feature in a female with single delivery compared to females under multiple deliveries. Therefore, the current study aims to compare the prevalence and severity of diastasis recti in primipara and multipara with FTND

Key results: Diastasis recti, Pregnancy, Full term normal delivery, Primipara, Multipara, Full term normal delivery

Limitation: It might be difficult to convince patient for being a part of this study.

Generalisability: Study not done yet.

Conflict of Interest: There is no conflict of interest

Source of Funding: No funding needed

Ethical Clearance: Being an observational study institutional ethical committee permission was taken.

References


Does Dermatoglyphics An Essential Tool for Predicting Dental Caries? - A Systematic Review

Suganya P1, Lubna Fathima1, Prabu D2, Raj Mohan3, Bharathwaj4, M.R. Prashanthy1

1Post Graduate Student (MDS), 2Professor, 3Reader, 4Lecturer, Department of Public Health Dentistry, SRM Dental College, Ramapuram, Chennai, India

Abstract

Background: Dermatoglyphics is one the vital tool which helps to predict many systemic diseases including oral problems. Dental caries seems to be a major problem overall the globe. The best way to manage this condition is only early diagnosis.

Aim: This study aims to assess whether dermatoglyphics effective in predicting dental caries.

Materials and Method: A systematic review of controlled trials was performed. The data were hand searched using electronic databases and the 328 number of articles were screened. The intervention and outcomes were assessed in the study included for systematic review. The bias assessment of the articles was done by using Newcastle Ottowa scale.

Results: Seventeen cross-sectional studies were included in our systematic reviews. Of that sixteen articles were found to be statistically significant, but further studies should be done in other countries to prove the association between dermatoglyphic pattern and dental caries.

Conclusion: The pattern of dermatoglyphics was found to be very effective in predicting dental caries and thereby prevent the caries formation at an incipient stage.

Key Words: Dermatoglyphics, Finger print, Dental caries, Streptococcus mutans.

Introduction

Dermatoglyphics or palmistry is the study of the permanent dermal ridges of the hand and feet. The term dermatoglyphics is procured from Greek word which means skin carving. It is the pattern of imprints of the epidermal ridges of the plantar and palmer surfaces of both feet and hands. In simple term, it is considered as the study of finger prints. The finger print of human beings always remains unique throughout their life time and it will not change. It is a very essential tool for the preliminary investigations such as the cases with suspected or doubtful genetic basis [1,2]

In the recent times, the dermatoglyphics plays a crucial role in determining the future incidences of mystery to a perfect application on a scientific basis. Hence it is very useful for the health care professionals in detecting the diseases at an earlier stage [3].

The dermatoglyphics have been correlated with many systemic diseases due to the coexistence in the morphology of organogenesis and the structure of dermatoglyphics by their interlinked genetic countenance. The dermatoglyphics study is based on the finger ridges which was under the jurisdiction of genetic and environmental factors; hence they act as a reservoir of the early genetic and developmental anomalies [2,3].

Currently, the dermatoglyphics have been used to predict many oral diseases such as precancerous lesions, oral clefs, dental caries and many other conditions. Of these dental caries is one of the most predominant disease that exists globally and affects all age groups in
general. Though many recent advanced materials and technologies have development to determine and treat dental caries but the problem still persists [2].

Dermatoglyphic pattern is considered as a genetic marker to predict dental caries due to the similarity in the ectodermal origin of the epithelium of finger buds, enamel and the primary palate which was development during the same intrauterine period [3].

Various studies have been conducted regarding the association of dermatoglyphic patterns and dental caries. To clarify this in an appropriate manner, this present study aims to evaluate the association of dermatoglyphics and dental caries by conducting a systematic review.

**Materials and Methods**

In this study the systematic review of trials was carried out to evaluate the association between dermatoglyphic pattern and dental caries.

**SEARCH STRATEGY:** The articles related to the correlation between dermatoglyphics and dental caries were hand searched using electronic databases such as Prospero, Web of science, Cochrane library, Wiley online library, Grey literature, Science direct and PubMed. The articles were retrieved from each database based on the mesh representation.

**INCLUSION CRITERIA:**

1. Original articles
2. Articles with full text are included
3. Only cross sectional studies
4. In vivo studies

**EXCLUSION CRITERIA:**

1. Articles other than English language
2. Review articles, case control
3. In vitro and animal studies are excluded.

**Search engine:**

- Cochrane library
- Wiley online library
- Prospero
- Pubmed
- Science direct
- Grey literature
- Web of science

**Results**

The figure 1 shows about the articles eligibility for the study. Totally 328 number of articles related to the correlation of dermatoglyphics and dental caries were retrieved. 206 articles which outfit the eligibility criteria were excluded from the study. Out of that 122 articles with full text were assessed for eligibility of the study. Finally 16 articles with full text were qualified for the study.

![Figure 1: Flow diagram showing the number of studies identified, screened, assessed for eligibility, excluded and included in the systematic review](image-url)
<table>
<thead>
<tr>
<th>Author Name</th>
<th>Year</th>
<th>Place</th>
<th>Study Design</th>
<th>Patient Age</th>
<th>Origin Of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bazmi et al [4]</td>
<td>2013</td>
<td>India</td>
<td>Cross sectional</td>
<td>4-14 years</td>
<td>Study group: with dental caries Control group: without dental caries</td>
</tr>
<tr>
<td>Thakkar et al [5]</td>
<td>2014</td>
<td>India</td>
<td>Cross sectional</td>
<td>12 years</td>
<td>Group 1- DMFT score 0 Group 2- DMFT score 1-3 Group 3- DMFT score &gt;3</td>
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<tr>
<td>Agravat et al [7]</td>
<td>2014</td>
<td>India</td>
<td>Cross sectional</td>
<td>5-12 years</td>
<td>Study groups: children with dental caries Control group: caries free children</td>
</tr>
<tr>
<td>Vijender et al [8]</td>
<td>2015</td>
<td>India</td>
<td>Cross sectional</td>
<td>5-12 years</td>
<td>Study group: DMFT &gt;5 Control group: DMFT=0</td>
</tr>
<tr>
<td>Sanghani et al [9]</td>
<td>2016</td>
<td>India</td>
<td>Cross sectional</td>
<td>6-13 years</td>
<td>Study group: caries active Control group: caries free</td>
</tr>
<tr>
<td>Deepti et al [10]</td>
<td>2016</td>
<td>India</td>
<td>Cross sectional</td>
<td>6-12 years</td>
<td>Hand prints of subjects were recorded</td>
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<tr>
<td>Saxena et al [11]</td>
<td>2016</td>
<td>India</td>
<td>Cross sectional</td>
<td>12-15 years</td>
<td>Group 1- DMFT=0 Group 2- DMFT 1-0 Group 3- DMFT &gt; 3</td>
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<td>Singh et al [12]</td>
<td>2016</td>
<td>India</td>
<td>Cross sectional</td>
<td>2-6 years</td>
<td>Group 1- dmft score 0-2 Group 2- dmft score 3-4 Group 3- dmft score &gt; 5</td>
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<td>Study Type</td>
<td>Age Range</td>
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<td>Maroli et al [13]</td>
<td>2016</td>
<td>India</td>
<td>Cross sectional</td>
<td>5-12 years</td>
<td>Group 1- caries free male children with DMFT score=0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>study</td>
<td></td>
<td>Group 2- caries free female children with DMFT score=0</td>
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<td>Group 3- caries active male children with DMFT score &gt;5</td>
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<td></td>
<td>Group 4- Caries active female children with DMFT score &gt;5</td>
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<tr>
<td>Elkawady et al [14]</td>
<td>2016</td>
<td>Egypt</td>
<td>Cross sectional</td>
<td>3-6 years</td>
<td>Study group- caries active children with dmft = 4</td>
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<td>study</td>
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<td>Control group- caries free children</td>
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<tr>
<td>Kaur et al [15]</td>
<td>2018</td>
<td>India</td>
<td>Cross sectional</td>
<td>6-12 years</td>
<td>Group 1: subjects with presence of ≥4 dental caries teeth</td>
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<td></td>
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<td>Group 2: subjects with no dental caries</td>
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<td>Reddy et al [16]</td>
<td>2018</td>
<td>India</td>
<td>Cross sectional</td>
<td>6-16 years</td>
<td>Study group: Group A- visually impaired with equal number of with and without dental caries</td>
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<td>Group B- deaf and mute children with equal number of with and without dental caries</td>
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<td>Group C- mentally disabled children with equal number of with and without dental caries</td>
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<td>Control group: Group 1- children with dental caries</td>
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<td></td>
<td>Group 2- children without dental caries</td>
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<tr>
<td>Srilatha et al [17]</td>
<td>2018</td>
<td>India</td>
<td>Cross sectional</td>
<td>3-6 years</td>
<td>Study group: def &gt;5</td>
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<td>study</td>
<td></td>
<td>Control group: def = 0</td>
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<tr>
<td>Chand et al [18]</td>
<td>2018</td>
<td>India</td>
<td>Cross sectional</td>
<td>4-14 years</td>
<td>Case group- caries active children with def/DMF score &gt;5</td>
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<td></td>
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<td></td>
<td>Control group- caries free children with def/DMF score = 0</td>
</tr>
<tr>
<td>Matar EA [19]</td>
<td>2018</td>
<td>Egypt</td>
<td>Cross sectional</td>
<td>3-6 years</td>
<td>Group 1- dmfs score = 0</td>
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<td>study</td>
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<td>Group 2- dmfs score &gt; 5</td>
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<td>Author Name</td>
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<td>Patient Characteristics</td>
<td>Outcome</td>
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<td>---------</td>
<td></td>
</tr>
<tr>
<td>Bazmi et al [4]</td>
<td>300</td>
<td>300 children of aged 4-14 years were selected and are divided into two groups based on the presence or absence of dental caries and their hand prints were obtained.</td>
<td>There was a statistically significant difference was found between dermatoglyphics and dental caries among study and control groups.</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Thakkar et al [5]</td>
<td>183</td>
<td>183 school children of aged 12 years were selected from Mangalore and are divided into three groups based on the DMFT/dmft score using WHO Proforma then their finger print was obtained using Cummins and Mildo method.</td>
<td>The dental caries are more prone among children with whorl pattern</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Kochhar et al [6]</td>
<td>99</td>
<td>99 children of aged 12-14 years were selected and are divided into two groups. One group for caries assessment and the other group for gingival and periodontal examination which was measured using DMFT index and O-S index then their finger prints were recoing Cummins and Mildo method.</td>
<td>The children with whorl pattern are more susceptible to dental caries than children with loop pattern</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Agravat et al [7]</td>
<td>200</td>
<td>200 children of aged 5-12 years were randomly selected from schools of Ahmadaabad and they were divided into study and control groups based on the presence of dental caries which was assessed using def index. Then their finger prints were obtained based on Cummins and Mildo method.</td>
<td>There was a statistically significant relation was found between children with whorl pattern and dental caries.</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Vijender et al [8]</td>
<td>100</td>
<td>100 children of aged 5-12 years were obtained from Government schools of Patiala and they were divided into two groups based on the presence or absence of dental caries which was recorded using DMFT index. Then their finger prints were obtained using Cummins and Mildo method.</td>
<td>Children with whorl pattern had increase number of dental caries whereas the children with loop pattern had lowest dental caries</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>
The 200 subjects of aged 6-13 years were selected and divided into two groups 100 children with caries active and 100 with no caries. Their hands prints were obtained and their dental caries were recorded using DMFT index. The caries active children had more number of whorl pattern whereas the number of loop pattern was found to be highest among caries free children <0.05

Samples of 300 children of aged 6-12 years were selected in Vadodara city, Gujarat and their hand prints were obtained. Their dental caries and malocclusion were recorded. There was a statistically significant difference between dermatoglyphics and dental caries < 0.03

A 276 number of children of aged 12-15 years were randomly selected from 6 private schools in Kanpur city and their finger prints were recorded using blue ink. Then their dental caries were assessed using DMFT index. The children with whorl pattern had more number of dental caries whereas the children with loop pattern are caries free <0.05

512 children of aged 2-6 years were selected randomly from schools in Lucknow and are divided into three groups based on the dmft score which was assessed using WHO Proforma, 1997 and their finger prints were obtained using stamp pad. Children with whorl pattern are more prone to dental caries <0.05

100 school children of aged 5-12 years were obtained and are divided into caries free and caries active groups with equal number of males and females in both groups. There was a strong association was found between dental caries and dermatoglyphics. Dental caries was found to be more common among children with whorl pattern especially among females Not specified

200 children of aged 3-6 years were selected from two kindergarten in Egypt and were divided into two groups based on the presence of dental caries using dmft index then their finger prints were recorded using stamp pad method. Caries active children had more of whorl pattern finger prints. The children with loop pattern have lower dental caries 0.000

**TABLE 2: Outcome of the studies based on the association of dermatoglyphic pattern and dental caries**
Cont. TABLE 2: Outcome of the studies based on the association of dermatoglyphic pattern and dental caries

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size</th>
<th>Details of the Study</th>
<th>Findings</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaur et al [15]</td>
<td>100</td>
<td>100 school children of aged 6-12 years were selected and divided into two groups equally based on the presence or absence of dental caries by using DMFT index. Their hand prints were obtained and their salivary pH were recorded using pH meter.</td>
<td>Children with dental caries had lower number of loop patterns and salivary pH levels when compared to those with absence of dental caries.</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Reddy et al [16]</td>
<td>300</td>
<td>300 numbers of children aged 6-16 years were selected randomly from schools in Tripati city and their finger prints were obtained using Cummins and Mildo method. The dental caries of these children was examined using ICDAS method.</td>
<td>The children with whorl patterns had more prevalence of dental caries when compared to other patterns in both case and control groups.</td>
<td>0.002</td>
</tr>
<tr>
<td>Srilatha et al [17]</td>
<td>100</td>
<td>100 children of aged 3-6 years were randomly selected and they were divided into two groups based on the presence or absence of dental caries which was assessed using def index. Then their finger prints were recorded using ink pad stamp method and their streptococcus mutans level were assessed using bacterial culture.</td>
<td>The children with whorl pattern had a higher of streptococcus mutans count.</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Chand et al [18]</td>
<td>100</td>
<td>100 children of aged 4-14 years were selected from Gujarat and are divided into two groups based on the presence of dental caries using def/DMF index. Then their finger prints were recorded.</td>
<td>There was a strong association was found between dermatoglyphics and dental caries</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Matar EA [19]</td>
<td>60</td>
<td>60 children of aged 3-6 years were selected in Egypt and were divided into two groups based on the dmfs score then their finger prints were recorded using Cummins and Mildo method.</td>
<td>Children with whorl pattern are more prone to dental caries whereas children with loop pattern are less susceptible to dental caries</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 3: Bias Assessment

<table>
<thead>
<tr>
<th>Author name</th>
<th>Selection</th>
<th>Comparability</th>
<th>Outcome/ exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample representation</td>
<td>Sample size</td>
<td>Non-respondents</td>
</tr>
<tr>
<td>Bazmi et al, 2013 [4]</td>
<td>*</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Thakkar et al, 2014 [5]</td>
<td>*</td>
<td>-</td>
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<tr>
<td>Kochhar et al, 2014 [6]</td>
<td>-</td>
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<tr>
<td>Agrawat et al, 2014 [7]</td>
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<tr>
<td>Vijender et al, 2014 [8]</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Sanghani et al, 2016 [9]</td>
<td>*</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Deepti et al, 2016 [10]</td>
<td>-</td>
<td>-</td>
<td>*</td>
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<tr>
<td>Saxena et al, 2016 [11]</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Singh et al, 2016 [12]</td>
<td>*</td>
<td>-</td>
<td>*</td>
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<tr>
<td>Maroli et al, 2016 [13]</td>
<td>-</td>
<td>-</td>
<td>*</td>
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<tr>
<td>Elkwatehy et al, 2016 [14]</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Kaur et al, 2018 [15]</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Reddy et al, 2018 [16]</td>
<td>*</td>
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<td>*</td>
</tr>
<tr>
<td>Srilatha et al, 2018 [17]</td>
<td>-</td>
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</tr>
<tr>
<td>Chand et al, 2018 [18]</td>
<td>*</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Matar et al, 2018 [19]</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
</tbody>
</table>

*Denotes low risk bias, - denotes high risk bias

Discussion

Dental caries is one the complex microbial disease that predominantly spreads throughout the world. The dental caries was caused by many factors but the exact reason is unknown. Even though many advanced materials have been developed for the treatment of dental caries, the best approach for the dental caries is prevention. To prevent the disease, the early detection of the root cause or the risk factor of disease is most important. Dermatoglyphics plays a very crucial role in
detecting many systemic diseases such as dental caries.

The study conducted by Sanghani et al in the year 2016 had discussed about the correlation of dermatoglyphic pattern among 200 school children of aged 6-13 years in India and they were divided into two groups equally based on the presence of dental caries then their finger were recorded. The children with whorl pattern have more prevalence of dental caries when compared to those children with loop pattern and it has found to statistically significant (P<0.05) [9].

Kaur et al in the year 2018 had discussed about the pattern of dermatoglyphics among 100 children with dental caries of aged 6-12 years in India and were divided into study and control group. The children with DMFT score> 4 were selected as study group and those children with no dental caries were choosen as control group then their salivary pH and finger prints were recorded for analysing the correlation of dermatoglyphic pattern and dental caries and concluded. The children with loop patterns was found to be more in the control group than the study group and it was found to be statistically significant (p<0.001) [15].

Bazmi et al in the year 2013 had discussed about the association of dermatoglyphics and dental caries among 300 children of aged 4-14 years in India and concluded that there was a strong association was found between dental caries and dermatoglyphics pattern. The children with whorl pattern had more dental caries when compared to children with loop pattern and it was found to be statistically significant (p<0.001) [4].

The study conducted study conducted by Deepti et al in the year 2016 had discussed about the association of dermatoglyphics between dental caries and malocclusion among 300 school children of aged 6-12 years in India and concluded that the children with increased dental caries had a greater frequency of loop pattern when compared to other pattern (p<0.03) [10].

Reddy et al in the year 2018 had discussed about the association of dermatoglyphic pattern and dental caries among 300 special children of aged 6-16 years in India and concluded that the children with dental caries had more frequency of whorl pattern whereas the children without dental caries had more frequency of loop pattern and it was found to be statistically significant (p=0.002) [16].

Saxena et al in the year 2016 had discussed about the correlation of finger prints and dental caries among 276 school children of aged 12-15 years from randomly selected schools in Kanpur city, India and they were divided into three groups based on the DMFT scores. The study concluded that the whorl pattern was most common among caries active group whereas the loop pattern was common among caries free children and it was found to be statistically significant (p<0.05) [11].

Srilatha et al in the year 2018 had discussed about the dermatoglyphic pattern and their correlation with dental caries by evaluating the streptococcus mutans level among 100 children of aged 3-6 year old children in India and concluded that the level of streptococcus mutans was found to be highest among children with whorl pattern. There was a statistically significant relation was found be whorl pattern and streptococcus mutans level (p<0.05) [17].

Agravat et al in the year 2014 had discussed about the efficiency of the dermatoglyphic pattern in the prediction of dental caries. The study was conducted among 200 children of aged 5-12 years from various schools in Ahmedabad, India and concluded that there was a statistically significant relationship was found between finger prints of whorl pattern and dental caries (p<0.05) whereas the children with loop patterns are caries free [7].

Vijender et al in the year 2014 had discussed about the effectiveness of the dermatoglyphics in determining the presence of dental caries among 100 government school children of aged 5 and years in Patiala, India and concluded that the dermatoglyphic pattern was very effective in predicting dental caries and there was a strong positive correlation was children with whorl pattern and dental caries (p<0.05) [8].

Elkwatehy et al in the year 2016 had discussed about the efficiency of the pattern of the dermatoglyphics in detecting the early childhood caries among 200 children of 3-6 years in Egypt and they were divided into two groups based on the presence or absence of dental caries. The study concluded that the dermatoglyphic pattern was very effective in predicting early childhood caries and it was found to be statistically significant (p=0.00),
The children with dental caries had highest frequency of dental caries whereas the loop pattern was found to more in caries free children\[14\].

The study conducted by Chand et al in the year 2018 had discussed about the cost effectiveness of dermatoglyphics in predicting dental caries among 100 school children of aged 4-14 years in Gujarat, India and are divided into two groups based on the presence or absence of dental caries. The study concluded that the dermatoglyphic pattern was very effective in predicting dental caries and it was found to be statistically significant (p<0.05)\[18\].

Maroli et al in the year 2016 had discussed about the dermatoglyphic pattern and its effectiveness in predicting dental caries among 100 school children of aged 5-12 years in India. There was a strong correlation was found between dermatoglythic pattern and dental caries\[13\].

Kochhar et al in the year 2014 had discussed about the pattern of dermatoglyphics and its association with dental caries and Periodontitis among 90 children of aged 12- 14 years in North India and are divided into groups caries assessment group and periodontal assessment group. The study concluded that the children with whorl pattern has a significant increase of dental caries (p<0.005) whereas the children with loop pattern had less dental caries and there was no significant relationship was found between dermatoglyphic pattern and periodontal diseases\[6\].

Matar et al in the year 2018 had discussed about the association of dermatoglyphics and early childhood caries among 60 children of aged 3-6 years in Egypt and are divided into two groups equally children with dental caries and children without dental caries. The study concluded that there was a statistically significant relation was found between the two groups (p<0.001). The children with whorl pattern are more common in children with dental caries and the loop pattern was common in caries free children\[19\].

Singh et al in the year 2016 had discussed about the correlation of dermatoglyphic pattern and dental caries among 512 preschool children of aged 2-6 years in India and concluded the children with whorl pattern had more dental caries when compared to other patterns and are found to b e statistically significant (p<0.05). Another study conducted by Thakkar et al in the year 2014 had discussed about the relationship between dental caries and dermatoglyphics among 183 school children of aged 12 years in Mangalore, India by obtaining their finger prints and the dental caries status and concluded that there was a statistically significant relationship was found between children with whorl pattern and dental caries (p<0.05)\[12,5\].

Overall analysis of the study shows that the dermatoglyphics is a best cost effective tool in predicting dental caries. This will be most helpful to both the health care professionals and the public in general for easy and early identification of the dental caries especially among mentally disabled children. Moreover it is very cost effective when compared to other diagnostic instruments and was very useful to identify and prevent the dental caries.

**Limitations:**

Only cross sectionals have taken into an account. Many studies which outfits the criteria have been excluded. Other databases should also be considered. Many studies have conducted only in India further studies should be carried out in other countries to get more relevant outcome.

**Conclusion**

Dermatoglyphic pattern was very effective in predicting the dental caries and helps the people to detect and prevent the dental caries at an initial stage. The finger prints with whorl pattern are more prone to dental caries whereas the finger prints with loop pattern are less susceptible to dental caries.

**Acknowledgement:** Nil

**Conflict of Interest:** Nil

**Ethical Clearance:** Taken from the Institutional review committee of Public health dentistry department, SRM dental college, Ramapuram.

**Source of Funding:** Self

**References**

1. Veeresh T, Mujahid A, Deepu P, Sivaprakash R. Correlation between Dermatoglyphics, Dental


Study on the Effectiveness of Abhaya Sunthi Churna and Snehana Swedana in Tamaka Shwasa

Suraj Sankh¹, Jyothy Kothanath.Bhaskaran²

¹P.G.Scholar, ²Head and Associate Professor, Department of Kaumarbhartiya, Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H), Datta Meghe Institute of Medical Sciences, Wardha

Abstract

Background: In Ayurveda Shwasa is a disease of Pranvha srotasa which is explained by all the Acharya in detail. The disease, Tamaka Shwasa in the contemporary system of medicine resembles the clinical features of Bronchial Asthma such as dyspnea, cough and chest discomfort. The etiological factors like exposure to smoke, dust, seasonal variation and exposure to cold are also similar in both the condition. A rough estimation in India shows 10-15% of Bronchial Asthma in the age group of 5-11 year old children.

Aim: present study is entitled to study the effect of Abhaya Sunthi Churna and Snehana, Swedana in management of Tamaka Shwasa. Material & Methods: The present study was designed as the comparative parallel group, interventional study, in which minimum of 30 patients were enrolled. The selected participants were assigned to the two observational groups. Each interventional group participants were administered with Abhaya sunthi churna for 14 days in both the groups A and B. Also in Group A there were additional Snehana and Swedana procedures for 7 days and in Group B consist of Churna with Conventional therapy.

Results: In comparison significant results were seen only in Kasa in group A whereas other parameters showed insignificant difference between the groups. The parameters such as Peenasa, Ghurghurkatwa, Kaphanishtivana, Astitvastha, Asinolabhesa, and Shwasakrichrrata showed reduction individually. The comparative result of both the groups on Adventitious Sound, Significant result was noted on the 14th day in group A who received Abhaya Shunthi Churna with Snehana Swedana after treatment in comparison with group B. Conclusion: we can conclude that the Symptoms of Tamaka Shwasa were reduced in both the groups (A and B) but the effect of the Abhaya sunthi churna with Snehana, Swedana i.e Group A was more significant than Group B.

Keywords- Tamaka Shwasa, Bronchial Asthma, Abhaya Sunthi Churna, Shwasa roga

Introduction

In Ayurveda Shwasa is a disease of Pranvha srotasa which is explained by all the Acharya in detail. As explained in Ayurveda with all the details of etiopathology, this disease entity stands as a very grave one as neglecting it would even cause serious complications and even death. As vital in adults it has also been specified with the same importance in children too as evident from the details of Shwasa roga given in Kashyapa Samhita, the compendium of Kaumarabhritiya. In this, Shwasa in children has been mentioned with the premonitory features in the view of early diagnosis in young children [1].

Tamaka Shwasa is one among the five types of Shwasa roga explained in all the classical textbooks of Bruhatrayee (Ayurveda)²,³. It is a disease which is said to be Yapya (manageable) and if it is of recent onset then it is Sadhya (Curable) [4].
The disease, Tamaka Shwasa in the contemporary system of medicine resembles the clinical features of Bronchial Asthma such as dyspnea, cough and chest discomfort. The etiological factors like exposure to smoke, dust, seasonal variation and exposure to cold are also similar in both the condition. Bronchial Asthma is defined as a chronic inflammatory disorder of airways which is associated with airway hyper-responsiveness. It leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or early morning[5].

It is said that this disease also causes burden on the number of lost school days and interferes with academic achievement and social interaction. A rough estimation in India shows 10-15% of Bronchial Asthma in the age group of 5-11 year old children. It also increases the number of preventable hospital emergency visits and admissions[6]. The chronic disease affects an estimated 4.8 million children[7]. According to WHO by the year 2020 Asthma along with chronic obstructive pulmonary disease will become the third leading cause of death[8]. According to Indian Survey, Prevalence regarding from heavy traffic region area in school going children showed prevalence 19.34%, but children of low socio-economic population is 31.14%,While the ratio of the children from low traffic area had 11.15% respectively and lastly in the rural area also showed 5.7% aged 6-15 years[9].

Ayurveda has various treatment modalities for Tamaka Shwasa which includes both internal and external therapies. Two types of therapies are advised which are Shamana (Pacification) and Shodhana (Purification). The churna of Abhaya (Terminalia Chebula) and Sunthi (Zingiber officinalis) is explained in treatment of Shwasa roga as a Shamana (Pacification) therapy[10]. Abhaya or Haritaki acts as anulomana (facilitating downward movement of Vata) and Sunthi acts as Pachana (Digestive), Kapha-Vataharana[11]. Other therapy is Shodhana (Purification) which have Snehana (Oilation) and Swedana (Sudation) over chest region have been explained for Tamaka Shwasa by Acharya Charaka[12] and Swedana has also been explained as one of the beneficiary procedure in Tamaka Shwasa[13].

Only a few research studies have been carried out on Tamaka Shwasa in children. Thus the present study is entitled to study the effect of Abhaya Sunthi Churna and Snehana, Swedana in management of Tamaka Shwasa.

Objectives of the Study

To study the effect of Abhaya Sunthi Churna on the clinical features of Tamaka Shwasa

To study the effect of Abhaya Sunthi Churna along with local Snehana Swedana on the clinical features of Tamaka Shwasa

Material and methods

Selection of material: The Raw drugs i.e Haritaki and Sunthi were procured from a local shop and authenticated by the Department of Dravyaguna, M.G.A.C.H. and R.C. Salod (H) Wardha.

Source the drug: The trial drug Abhaya sunthi churrna was prepared at Rasashastra Department in the college as per the classical method of Churna preparation.

Preparation of material: Ingredients were cleaned properly and taken in equal quantity and dried under sunlight. Each ingredient was pounded to fine powder by Pulverizer (Mesh no.80) & sieved, Each ingredient was weighed separately, Mixed together and finally the drug was prepared and made ready by packing in the air tight container in which for age criteria 3-9 years 100 grams of Churna was packed and for 10-15 years 200 grams of Churna was packed in the air tight container.

Methods

After receiving approval for research from Institutional Ethical Committee Ref no DMIMS(DU)/IEC/2017-18/7247 the comparative parallel group, interventional study was conducted. Total 30 Children aged 3 – 15 years with clinical symptoms and auscultations of Tamaka Shwasa were enrolled from OPD & IPD, Department of Kaumarbhritya of M.G.A.C.H. and R.C. Wardha.

The selected participants were assigned to the two observational groups. Each interventional group participants were administered with Abhaya sunthi churna for 14 days in both the groups A and B which was decided following textual reference. Also in Group A there were additional Snehana and Swedana procedures.
for 7 days and in Group B consist of Churna with Conventional therapy if the participant is on any kind of conventional therapy. Detailed history of the participant was taken from the informant and the participant too with thorough general and systemic examination as per the parameters mentioned in the case Proforma which was prepared for the clinical trial of the study. From the 1st day itself the observations and laboratory sampling were recorded before and after the treatment. Hence the observational results were analyzed by statistical tests and post treatment follow up was done on 28th day.

**Administration of the trial drug:** The trial drug, Abhaya sunthi churna was administered in the following dosage for a period of 14 days.

**Table no.-1: Dosage of drug as per age**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Drug doses</th>
<th>Aushadha Kala</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 – 9 years</td>
<td>06 grams/day</td>
<td>4 times a day</td>
</tr>
<tr>
<td>10 – 15 years</td>
<td>12 grams/day</td>
<td>4 times a day</td>
</tr>
</tbody>
</table>

**Inclusion Criteria:**

- Diagnosed participants of TamakaShwasa between the age group from 3 to 15 years of age irrespective of caste, religion, sex, habits, occupation and socio-economic status

**Exclusion Criteria:**

- Severe persistent Asthma which needs usage of nebulization and inhalation of nasal spray (puffs).
- Diagnosed cases of Severe COPD, Respiratory Distress Syndrome (RDS), Pneumonia, Pulmonary Kochs and AIDS, Status Asthmaticus
- Any other systemic disorders which may interfere with the trial.

**Diagnostic criteria-**

A). Subjective criteria:

- The assessment was done on the basis of Classical signs and symptoms of tamaka shwasa
  - Peenasa (Coryza)
  - Ghurghuratwa (Wheezing)
  - Kasa (Cough)
  - Kapha Nisthivana (Expectoration)

- Asino Labhate Saukhyam (Relieving while sitting position)
- Shwaskrichhrata (Difficulty in Breathing) related to climate
- Nidra (Sleep)

B) Objective criteria:

- Adventitious Sound
- CBC (Complete blood count)
- A.E.C (Absolute Eosinophil Count)

**Result and Observations**

As per the demographic data maximum numbers of participants were from age group 8-13 years. Age is often considered as a risk factor for Bronchial Asthma because it is unclear if malnutrition status leads to Bronchial Asthma or if the age reflects the sum of cumulative response throughout the life. Ageing of the airway and parenchyma may lead to structural changes associated with Childhood Asthma. Out of 30 participants, males were 12 in Group A and 7 in Group B whereas females were 3 in Group A and 8 in Group B. The ratio of males is seen more which was just serendipity (coincidence) rather than any logic behind this observation.
Distribution according to area of residence revealed 53.3% of participants were from rural area in both the Group A and group B, followed by 46.7% from urban in both the Group A and B. In the past most studies have reported that prevalence and mortality rate Bronchial Asthma are greater in urban area rather than rural area because of the reflecting change and patterns of the environment and climate in developing and developed countries. All this is worsening due to one major causes i.e. pollution in urban areas which is affecting a big threat of global warming. In the present study, the site was a rural area and the population was also from the same area. The prevalence of Bronchial Asthma even in rural areas suggests that environmental pollution might have started influencing even the rural areas. Even the nearby industries might be contributing to the disease in children. Burning wood, cow dung, crop residues and coal in open fire is typically found in the location of the study which might have also contributed to this observation.

On the basis of religion, nothing specific can be predicted from the observation in the present study, as the demographic area might have played the major role in it. With respect to the status of education, all the participants were school going children and there is no such fact which relates prevalence of Bronchial Asthma in school going children.

Disturbances in sleep are an essential feature quoted in the lakshana of Shwasa in Charaka Samhita. In the present study, sound sleep was found in 53.3% of participants in Group A and 73.3% of participants in Group B which is suggestive that the sleep disturbances may not be an essential feature of Tamaka Shwasa in children as in adults. Disturbed pattern of sleep in Group A was 7 (46.7%) participants and only 4 participants in Group B. This might be because in children, sleep pattern is not considered as the major problem.

Around 10 (33.33%) participants were found to be having a familial history of similar condition and hence this observation also did not show any obvious relation. Noticeably Nidana explained for Tamaka Shwasa in Ayurveda do not include hereditary factors involved in the pathology. But as per modern medicine, significant risk has been observed in participants who frequently suffer from uncontrolled asthma symptoms, Severe Exacerbations in previous year, Exposure to potential allergens/triggering agents and Poor adherence to past treatment.

15 participants in each group were having a vegetarian and mixed diet. Majority of the participants were having the habit of consuming Ruksha Aahara like chips, packet food and fried items. As these food items are meant to cause Vata prakopa and are among the causative factors of Shwasa roga, it can be said that there is a direct relation between the type of food consumed and the disease manifestation.

Subjective Parameters

In comparison significant results were seen only in Kasa in group A whereas other parameters showed insignificant difference between the groups. The parameters such as Peenasa, Ghurghurkatwa, Kaphanishtivana, Asinolabhatesaukhya and Shwasakrichrrata showed reduction individually.

Objective Parameters:

In the comparative result of both the groups on Adventitious Sound Significant result was noted on the 14th day in group A, who received Abhaya Shunthi Churna with Snehan Swedana after treatment in comparison with group B.

Result on Hematological parameters as shown below:

In comparison the result of both the groups in Objective parameters insignificant results in the values of White blood cells at the end of the trial was seen between Group A and B with a p value of 0.547.

In the values of Monocytes, the comparative result was insignificant statistically. But in the values of Granulocytes, Lymphocytes and Basophils significant results were seen on comparison between Group A and B with a p value of 0.001, 0.001 and 0.003 respectively. In the values of Eosinophils and Absolute Eosinophil Count there were no significant results seen on comparison between the groups.

Overall assessment of the result:

Overall maximum improvement was noted on Subjective Parameters of Tamaka Shwasa in Group A
with 77.98% and Moderate improvement in Group B with 62.85%.

**Discussion**

In the present era, children are commonly seen suffering from Childhood Asthma. Although both the conditions cannot be completely looked at as the same due to differences in etio-pathological factors explained in Ayurveda, clinical correlation was the purpose in doing so. In the study Abhaya Sunthi Churna was selected as it was one among the formulations indicated in Tamaka Shwasa which contained easily available drugs. Availability of genuine herbs is becoming a grave issue day by day due to excess production of herbal products and scarcity of good quality herbs. So it was the need of the hour to have a simple but efficacious formulation in a condition like Tamaka Shwasa. Snehana Swedana being the Purvakarma are very widely practiced in many clinical conditions and are also indicated in Tamaka Shwasa during the vegavastha\(^\text{[14]}\)

In the present study, significant difference between the groups was found in only Kasa whereas other parameters showed insignificant results on comparison. All the participants in Group A had no cough on 14th day whereas 5 had occasional cough in group B. This significant effect in group A is suggestive of the enhanced action of Abhaya Shunthi Churna when administered with Snehan Swedan. The insignificant effect on comparison over Peenasa, Gherghuratwa, Kapha Nishtivana, Asinolabhate saukhyam and Shwasakrichhrata shows that both the groups were equally effective in the management of Tamaka Shwasa. When analyzing the individual percentage of relief, it is evident that the number of participants who got relieved of the symptoms were more in Group A than B. The insignificant difference between the groups may also be due to the less sample size in the present study.

In comparison with group B, group A who received Abhaya Shunthi Churna with Snehan Swedan showed significant results after the treatment i.e. on the 14th day on the objective parameter of Adventitious sounds. 14 participants out of 15 had no adventitious sounds on auscultation in group A which shows better efficacy of the treatment modality. The pathology of Shwasa does not involve only systemic changes but localization of Kapha Dosha in ura pradesha. Kapha vilayan with internal medicine always gets enhanced by the local effect of Snehan Swedana because of which these procedures have been mentioned specifically in the management of Tamaka Shwasa. The same efficacy was seen in the present study which indicates the necessity of local procedures in early relief from the symptoms.

On the basis of mean of before and after treatment significant effect was seen in the values of Granulocytes the value of which was 42.93 on day 1 but was 60.27% on day 14 in Group A. As the level of increase was within the normal limits no specific effect of the drug can be ascertained from the values. Larger sample studies might earn better conclusions. Although there was a significant difference between the effect of both the groups, the changes in the values of Lymphocytes was within the normal limits. The same was seen in the values of Basophilhs also. Although there was reduction in the values of Eosinophils and Absolute Eosinophil Count in both the groups, the difference was not significant in the study. Larger sample evaluation might give precise results. No Adverse Drug Reaction (ADR) reported during the follow up period reveals that the drugs did not cause any untoward effect in the participants.

**Probable mode of action**

In the literature of Ayurveda, the main causes of Tamaka Shwasa are the vitiation of Vata and Kaphadosha including the formation of Ama in which the drugs having the properties of Deepana, Pachana, Vata Anulomana, Shwasahara, Kasahara and tridoshahara can break the Samprapti of Tamaka Shwasa.

Tamaka Shwasa has the pathological state of Vata and Kapha Dosha in dominance. Pediatric age group has been explained in Ayurved classics as a period where there is physiological dominance of Kapha Dosha. The churna of Abhaya (Terminalia Chebula) and Sunthi (Zingiber officinalis) is explained in Shwasa chikitsa of Bhaisajya Ratnavali\(^\text{[15]}\). Abhaya or Haritaki acts as anulomana (facilitating downward movement of Vata) and Sunthi acts as Pachana (Digestive) and Kapha-Vata hara\(^\text{[11]}\). Haritaki is having immunomodulatory activity which directly has its effect on Pranavaha Srotas\(^\text{[16]}\) Sunthi acts as Antitussive in the management of Tamaka Shwasa\(^\text{[17]}\).
In this study, two modalities of interventions which are indicated in the management of Tamaka Shwasa have been compared in the same set of participants. One group was administered with only internal medication as in the routine management protocol whereas the other group was given Snehana and Swedana therapies which are a very unique contribution of Ayurveda in the management of Shwasa roga. Snehana was performed with Tila Taila (Sesame oil) which is said to be having the properties of Ushna virya, Vata shamaka and not increasing Kapha Dosha\(^{[18]}\) By virtue of its effect, Snehana might have helped in the normal movement of Vata which was extended by the added effect of Swedana. Kapha vilayana might be the specific effect of Swedana by virtue of its Ushna guna. This formulation also facilitated the normal downward movement of Vata with respect to respiration which reduced the gravity of dyspnea and difficulty in sleep. The reduction of excess quantity of sputum brought about by the drugs used helped in listening to the adventitious sound on auscultation of chest.

**Conclusion**

In the present study Tamaka Shwasa was correlated with the Childhood Asthma or Bronchial Asthma. In children Consumption of Ruksha Aahara, packet foods and Agnimandya were the major etiological factors. During the study the Abhaya sunthi churna was well tolerated by all the participants without any obvious Adverse Drug Reaction (ADR).

The results of the study revealed that the Symptoms of Tamaka Shwasa were reduced in both the groups (A and B) but the effect of the Abhaya sunthi churna with Snehana Swedana i.e Group A was more significant than Group B.

Hence it can be concluded that Abhaya sunthi churna with external Snehana and Swedana can be effectively used in children suffering from Tamaka Shwasa as compared to only oral administration of Abhaya sunthi churna for a period of 14 days.

**Ethical Clearance** is taken from Institutional Ethical Committee Ref no DMIMS(DU)/IEC/2017-18/7247.

**Conflict of Interest:** NIL

**Funding:** Self

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A Study Protocol for Checking Efficacy of Leap Motion Device on Gross Hand Dexterity in Sub-Acute Stroke Patients

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Abstract

Introduction: Stroke survivors have limited everyday living tasks, often because of dexterous issues. Videogame-based training (VBT) with the appearance of virtual reality helps to improve the role of the upper limbs. The leap Motion controller can track both hands and fingers’ fine movements. The studies thus demonstrate the efficacy of the leap motion system on gross hand dexterity in patients with sub-acute stroke.

Method: The research has been designed as an experimental study. The total of 40 participants will be taken from AVBRH, sawangi Meghe for study as per inclusion and exclusion criteria. With intervention the span of the study will be 6 months. Leap Motion-based, augmented reality training will be provided to patients for half hour, every single day, Five days of the week a month. Pre and post, box and block test and System usability scale will be taken. Those two will be the patient’s major outcome.

Discussion: This study protocol aims to assess the efficacy of the leap motion controller on the rehabilitation of gross hand dexterity in patients with stroke. The study’s expected outcome will concentrate on the evaluation of the usability of VBT using the Leap Motion Controller (LMC) to train gross hand dexterity in stroke patients’ early recovery process.

Keywords: stroke, upper limb, dexterity, virtual reality, videogame-based training (VBT), leap Motion controller (LMC), box and block test, system usability scale.

Introduction

1The World Health Organization (WHO) estimates that stroke is the second-largest cause of death in the world.2Up to 85 percent of patients who survive stroke hemiparesis due to reduced arm and hand function.3The upper limb is an important part of the human body, which is very mobile and plays a role in grasping, carrying, moving and touching different objects.4Loss of upper limb independence contributes significantly to physical disability, impacting quality of life and independence in the ‘basic’ and ‘instrumental’ everyday activities.5To further boost the outcome of the upper limbs, work is continuing to explore new approaches. Videogame-based training (VBT) together with virtual reality (VR) is an emerging therapeutic process. Recent meta-analysis suggests there is strong evidence that VR programming can be helpful for upper limb recovery following stroke.

Virtual reality training is becoming a promising technology which can facilitate motor rehabilitation by providing high-intensity, repetitive and task-oriented training with computer programs that simulate three-dimensional scenarios in which patients play by moving their body parts.6Virtual Reality (VR) may provide patients with a supportive immersive experience, with the goal of maintaining high quality and intensive
Within the literature, the following benefits of using VR within rehabilitation have been identified in improving performance, improving the affected limb and cognitive functions, encouraging neuroplasticity and greater flexibility in everyday life activities, as well as increasing patient engagement and cooperation during rehabilitation programmes. Indeed, some authors have found evidence that using virtual reality and immersive video games can be helpful in improving upper limb function and function of ADL (Daily Living Activities) when used as an alternative to regular therapy (to maximize total therapy time) or when compared to the same typical therapy dose. This makes VR a valuable resource in therapy’s future, not only because it has been shown to be effective for ill and healthy subjects, but also because it has had very little side effect and was much safer than other aggressive or violent therapies.

Leap Motion’s leap motion controller (https://www.leapmotion.com) provides a means to capture and track delicate hand and finger movements, while manipulating a virtual environment that involves hand-arm coordination as part of virtual tasks. The Leap Motion Controller (LMC) is a modern optoelectronic device designed to capture both hands’ movement and manipulate a virtual environment. Unlike previous devices, it optoelectronically monitors fine finger movements, using neither glows nor markers. This system is specifically designed for hand gesture recognition and explicitly measures the location of the fingertips and the orientation of the hand. This is made with 2 cameras and 3 LEDs in infrared. Across three-dimensional space it senses hands, wrist and elbow positions and gestures. LEAP motion setup is quick, since it only requires downloading the SDK from the official LEAP motion website.

Aims and Objective

This research aims to assess the efficacy of the leap motion controller on the rehabilitation of gross hand dexterity in patients with stroke.

Objectives

1. To demonstrate the effect of Virtual Reality based serious gaming using leap motion the dexterity of hand in patients with strokes.

2. To check the usability of VBT to train gross motor function in the upper limb hand function using Leap Motion Controller (LMC).

Methodology

This study will be conducted in the Department of Community Health Physiotherapy at Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, India, with the approval of Datta Meghe Institute of Medical Sciences, Institutional Ethics Committee, Deemed to be University.

Study Setting: - Ravi Nair Physiotherapy College

Study Type:- Experimental study

Study DuratioN:- 6 months

Sample Size:- 40

Inclusion Criteria:-

1. Patient willingness to participate
2. Age 30-70 years old
3. Those who rated stage 4 or above in the stages of arm and hand recovery in the Brunnstrom motor.
4. Those who mastered the doctor’s guidance in Mini-Mental State Evaluation, with or above 24 marks.
5. About 4–24 weeks had passed since the onset of the stroke.
6. Capable of raising the affected arm and wrist independently of their residual voluntary movement or at least capable of carrying out an ante-flexion of their upper arm and length of one or more fingers against gravity

Exclusion Criteria:-

The exclusion criteria are as follows:

1. Non-willing to participate
2. Recent fractures
3. Visual, hearing deficits
4. On Brunnstrom grading patient score 1.5 or
5. The background of the ischemic transient
attack (TIA);

6. Vital body parts including brain, lung, liver and kidney failure;

7. Past neurosurgery or epilepsy experiences inside the brain;

8. Significant cognitive disability or aphasia (incapable of following the directions of the therapists);

9. Not appropriate for MRI scanning (including but not limited to: metal pieces in the eyes or face; insertion of any electronic devices such as (but not limited to) cardiac pacemakers, cardiac defibrillators, cochlear implants or nerve stimulators; operation on brain blood vessels or heart valves; or defects in the brain or skull);

10. participation in another clinical trial concerning the treatment of physical or investigational medicines.

OUTCOME MEASURES:-

1. The dexterity will be measured using the box and block test.

2. 5-point Likert scale for System Usability Scale.

PROCEDURE:-

The game is played with both hands beginning with non-affected first hand. Participants should sit on a chair with a rectangular pillow on their lap (if not in a wheelchair), so that the elbows can rest on the pillow. The LMC is positioned on a table in front of the person, between the body and the LED screen. During each session, the principal investigator will sit beside the participants, providing online feedback (if necessary) through verbal, visual and/or physical guidance. The LMC is comprised of three infrared emitters and two charged device cameras to monitor all movement of hands, wrists, and forearms. The infrared emitter light reflects back from the hands' surfaces so no markers are required.

Petal-picking-game:- Designed by picking lotus petals in a simulated environment to develop the finger pinching motor skills. This also strengthened digital dexterity and coordination.

Experimental procedure:- Leap Motion-based, augmented reality training will be provided to patients for half hour, Every single day, Five days of the week a month.

Material Required

- Printed copy of brunnstrom scale, mini mental scale, system usability scale
- Pillow
- Chair with arm rest
- Table
- Stopwatch.
- Wooden box dimensioned in 53.7 cm x 25.4 cm x 8.5 cm.
- 150 wooden cubes (2.5 cm in size)

INSTRUMENTATION:-

Leap motion controller
LED screen

Data Analysis

Analysis of data will be carried out using concise and inferensive statistics using unpaired t test students in chesquare. The software used in the study will be the SPSS 24.0 version, the paradox prism 7.0 version and the degree of significance < 0.005 (p>0.005 m) is considered.

Ethics And Dissemination

The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review.

Observation And Expected Results:-

The study’s expected outcome will concentrate on evaluation of VBT usability using the Leap Motion Controller (LMC) to develop gross hand dexterity in stroke patients’ early recovery process.

Discussion

This study protocol aims to assess the efficacy of
the leap motion controller on the rehabilitation of gross hand dexterity in patients with stroke. The purpose of this study will help to explain the process of creation of a series of VR mini-games designed to improve the encouragement of patients with stroke when performing repetitive upper limb movements. The study's expected outcome will concentrate on the evaluation of the usability of VBT using the Leap Motion Controller (LMC) to train gross hand dexterity in stroke patients’ early recovery process.

**Source of Funding:** There will be no direct support for this research from public and private organization. The department of physiotherapy, at Datta Meghe institute of Medical Science, Deemed to be university will provide material needed for research.

**Conflict of Interest:** Nil

**References**


A Novel Research Protocol to Evaluate Psychological Perception Using Brain Gym Exercises in Physiotherapy Students

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Abstract

Brain Gym is an academic kinesiological program that is promoted and applied with a consistent learning purpose that aims at enhancing performance such as memory, psychological perception and cognitive skills. The technique requires the participant to communicate with a series of activities that help the body to understand the primary behaviour and learn how to coordinate the brain and entire body. Brain Gym activities includes of 26 basic motions, which are believed to improve perception and stimulates brain hemisphere by neural re-modelling to facilitate whole brain learning. By ways of balancing both the side of brain, behavioral difficulties, social and intellectual burdens are expected to be reduced.

Aims and Objective: This study aims to evaluate the psychological perception and decrease depression in the undergraduate physiotherapy students.

Method: Here’s a idea we suggest to check the psychological perception with brain gym intervention and the duration for practices comprises of three days a week session, duration of 25 minutes, which is completed in one hour. The depression anxiety stress scale (DASS 21) is used to evaluate disorder which is a valid and reliable tool. This study will be conducted in Ravi Nair Physiotherapy college, Sawangi, Meghe, Wardha. The duration of the study will be six months. The study design is of before after-type with simple randomized sampling.

Result: The data will be analysed using Student paired t test.

Conclusion: The expected outcome includes the detection of stress, depression and anxiety levels which will be evaluated by using DASS-21. Data analysis will be done using students paired t test and conclusion of the study will be published after the results are analysed.

Keywords: Psychological perception, Depression, Stress, Anxiety, DASS-21, Brain gym

Background

It is an interventional type with before-after study design which focuses on the evaluation of the psychological perception after intervention of brain gym exercise. Brain Gym was developed in 1970 as an educational and psychological training system developed and enforced with a specific learning intent. Brain Gym activities includes of 26 basic motions, which are believed to improve perception and stimulates brain hemisphere by neural re-modelling to facilitate whole brain learning. The neural mechanism and white matter connectivity of the brain is influence by the intervention of the exercises.

Brain Gym is a instructional curriculum intended to improve social, mental, emotional and physical efficiency and use 26 moves. According to Brain Gym literature, the abstract framework on which brain activity is conceptualised is generally simplified and defined along dimensions: laterality, attention and centring. Laterality, the synchronization between the
brain’s right and left hemispheres, which is considered important for reading, writing, hearing, communicating and being able to walk and think. Focusing, the ability to process information in the brain, which is connected to perception and lack in attention / hyperactivity. The final section, centring, the top and bottom brain parts organized as necessary to combine rational thought with emotion (5). Brain gym intervention aims at the optimization of activity, social participation, and quality of life, as well as the health condition of people with acute and chronic disabilities. The most beneficial way to stimulate the brain is by incorporating kinesthetic and tactile learning, techniques and audio and visual activities to combine the high- and low-brain functions. Brain exercise contributes to sensory integrity, motor learning and a link between brain and body. Recently, brain-inspired methods have gained more popularity in overcoming command and decision-making challenges (6). A motion in the mental workout has been shown to have increased blood circulation and stability, good oxygen levels and healthy metabolism (7).

As per the founders, the daily practice of brain gymnastics leads to activation and development of various sections of the brain, particularly the cortex which allows for smoother and more organized communication between the two sides of the brain for high-level thinking (8). The brain is a complex organ which focuses on motion and according to Hannaford, “activity is necessary for learning” (9). Brain stimulation is very important in neuro-rehabilitation, reducing atrophy, lessen the risk of brain structure lesions and increasing cognitive performance. It is important therapy for elderly patients with depression, as its neuronal advantages have increased for age-related atrophy exacerbated by neuropathology (10). Neuroimaging research has indicated motor development, improve perception and integral approach involve stimulation of domains of auditory perception and more operation of cerebral cortex. The study describes the importance of brain gym exercise in physiotherapy. Exercise can stimulate the brain in such a way that neurons are often in a condition to handle the different data from outside and are capable of responding to a “corporate member” of their duty in compliance with parts of brain activity using the principle of “brain-body link”. Brain Gym is a great source of personal development, enabling individuals to obtain rapid transformations and also improve the quality of life in a different age group.

Many recent experiments have been carried out to determine the efforts required to enhance and stimulate the psychological perception of brain (11). Perception is defined as a essentially relational process in which visual stimuli is translated progressively into projections which serve as the basis for action (12). If a person is nervous or distressed then automatically the energy is pumped into the brain and the brain loses control, thus caused the primed, unexpectedly impaired reaction. Brain gym training can minimize mental stress and encourage brain concentration and perception (13). Students nowadays are under stress from waking up to not having proper sleep in addition to all the pressure of appraisals and examinations (14). This undoubtedly influences the attitude of the student towards leaning and academic success (4).

Dr. Chaitanya Kulkarni, Dr. Sanjivani Ramesh Khandale have done a project to detect the effects of brain gym exercise on the attention span of the young students and concluded that the mechanism of reading, recoding and comprehension has been improved. Also, the effect of these exercises demonstrated an improvement in eye power and hand control, as well as helping to focus on the same focal point while reading and writing concurrently. E Effendy, N Prasant Conducted a analysis demonstrating the effect of brain gymnastics on the population of Nursing Home Care Medan ‘s and found that brain gymnastics increased PSQI and HARS score levels in the intervention category and reduce stress and depression, in year 2019 (15) here they have found a significant change in life style and improved quality of life in the elderly patient. Keith J. Hyatt in year 2007, conducted a study on school students and finished with a segment explaining Brain Gym exercises to encourage literacy ability, oral reading comprehension, communication skills, pronunciation and learning, self-esteem, memory, analytical thought, imaginative thinking. Brain exercise has seen to be effective in attention improvement, in research and more on it is shown to be very helpful in enhancing concentration, attention, vision and memory as well as helping to relieve stress.

This research explores the impact on the psychometric characteristics of undergraduate students.
in the exercise program called as brain gym\textsuperscript{(16)}. Specially because it seems to be a accurate and easy-to-administer scale, the Depression Scale, Anxiety, and Stress-21 are chosen. The DASS-21 consist of three self-report measures used to assess depression, anxiety, and emotional tension\textsuperscript{(17)}. Depression spectrum tests dysphoria. Anxiety assessment tests autonomic activation, psychological anxiety\textsuperscript{(18)}. Stress is the very common condition faced by students nowadays and causes disturbance in their daily lives.

Thanh Duc Tran, Jane Fisher performed a study on the Efficacy of DASS-21 in a cohort of Northern Vietnamese people in rural communities as a screening tool and the study concluded that it can useful in clinical practice and the components such as depression, and stress levels are determined by summing the ratings for the elements in question\textsuperscript{(18)}.

The research aims to calculate the impact of brain gym exercise on stress, anxiety and depression in the student of undergraduate physiotherapy using the scale of DASS-21 as an evaluation method. The brain gym exercise intervention will be performed in all the subjects and then evaluation of the psychological perception will be conducted.

**Methodology**

The study will be conducted in Ravi Nair Physiotherapy College, after the approval the Institutional Ethics Committee (IEC) of Datta Meghe Institute of Medical Sciences, Deemed to be University, Sawangi (Meghe). The study design is of before after-type with purposive sampling.

**Study design**: Interventional study

**Sample Size**: 220

**Duration of study**: 6 months

**Inclusion Criteria**:  
- Age group - 18 to 23 years  
- Physiotherapy undergraduate students

**Exclusion Criteria**:  
- Not-willing to participate  
- Student with migraine headache  
- Diagnosed with psychological condition  
- History of neurosurgery or cognitive damage

**OUTCOME MEASURE**:  
- Depression, anxiety and stress scale (DASS-21)

- The DASS-21 is a valid and reliable instrument for determining the mental status, with reliability for depression = 0.81, anxiety = 0.89, stress = 0.78 respectively.

- The DASS-21 has sensitivity = 89% and specificity = 76%.

**NEED OF STUDY**:  
1. To calculate the psychological perception among undergraduate students in physiotherapy.  
2. To reduces depression, anxiety and emotional stress in undergraduate students.  
3. To assess the effects on psychometric properties in undergraduate students.

**PROCEDURE**:  

The Institutional Ethics Committee (IEC) Clearance will be obtained priorly. Students will be selected as per the inclusion criteria that has been mentioned. The participants will be informed the aim of the research and will get informed consent. They will be given pre and post interventional assessment using DASS-21 scale, reading will be recorded and the exercise intervention will be given for a month. After the results have been obtained, data collection will be done and statistical analysis will be obtained and the conclusion will be given and a research paper will be created according to the study and published.
**Brain Gym Exercises Treatment Protocol:**

The **Marching** exercise is performed at the beginning as warm-up, in which subjects stand straight and lift both the legs continuously slight above the hips for a time duration of 1 minute. A **Cross Crawl** exercise is performed to enhance the coordination between both the sides of the brain; it is done for a time duration of 2 minutes (5 sets of 8 repetitions). Subject is instructed to stand straight and lift up the leg up to the chest and touch the knee with the opposite elbow.

**Positive Points** helps to improve memory and reduce stress levels. The subject is instructed to breathe deeply and gently press the eyeballs with eyes closed for a time duration of 1 minute (10 repetitions). It helps to stimulate the lateral and side to side coordination.

A **Step Touch** exercise is done and is performed in the standing position and the subject is instructed to simultaneously move right legs toward left and left towards right, should be done for a duration of 5 minutes (30 repetitions).

A **Neck Circles** exercise helps to reduce stress on the neck muscle, head movement coordination and move the neck in circular motion for a time frame of 2 minutes (3 sets of 8 repetitions). A **Cook’s Hook-Up** helps it stimulates the neurons and enhances the balance between hand and brain. The subject is instructed to extend and cross both the hands and fix the fingers together and internally rotate the hands for a time duration: 5 minutes (repetition).
A Brain Button - This exercise is performed and helps to improve the flow of electromagnetic energy and, helps in relaxation. The subject is instructed to palpate belly button with one hand and other hand over the collarbone and perform circular motion with finger for a time duration of 2 minutes (10 repetition) (17). The Thinking Cap helps to enhance learning speed and mood, increase attention span, and improve memory. The subject is instructed to press the top of ear and the bottom continuously (3) duration: 1 minute (15 repetition) (24).

A Lazy Eight helps in boosting eye muscle control, balance, and concentration. The subject is instructed to extend the hand and make the figure of Eight horizontally in front (3) Duration: 1 minute (5 repetition). A Trace X helps to increase attention span and improve focus (25), where the subject to close eyes and imagine a figure of “X” and do eyeball movement the duration is 2 minutes (10 repetition).

Expected Result

Once the study is completed statistical analysis will be done using Student paired t test and presented in the form of research paper.

Discussion

The study protocol aims to evaluate the psychological perception in an undergraduate student by using the DASS-21 scale. We hypothesis that there will be improved ability to concentrate and improved focus. The research will help to prevaricate the effectiveness of brain gym exercise on stressed student and help with strategies involved to decrease stress and anxiety.

The DASS-21 is chosen as the aspects addresses the challenges faced by the current generation i.e. stress, anxiety and depression and it entirely evaluate the mental status of a person. The Adaptive practices for students include everyday life activities, effective speech, cognitive skills, adaptability, and learning skills that are observable and based on national norms.

Conclusion

The expected outcome includes the detection of depression and anxiety levels in Undergraduate physiotherapy students which will be done by using DASS-21. Brain gym exercises as an intervention will be taught to the participants to evaluate the psychological perception and solve their intellectual and behavioural challenge.

Ethical Clearance: Institutional Ethics Committee (IEC) of Datta Meghe Institute of Medical Sciences, Deemed to be University, Sawangi (Meghe).

Conflict of Interest: None

Funding support: None

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Original Research

Socio-demographic Profile of Deaths due to Poisoning at a Tertiary Care Hospital in Bangalore

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Abstract

Background: Deaths due to poisons form inexorable part of all unnatural deaths in the current era of social, cultural and personal dilemma existing among mankind. Stress, poverty, financial insecurities, personal conflicts, unemployment, ill health, psychological disturbances, difficulties in psycho-social adaptability and loneliness contribute significantly to the ever-increasing mortality in poison deaths.

Methods and results: A 20-month prospective descriptive study was conducted at the Department of Forensic Medicine and Toxicology, Victoria Hospital, Bangalore Medical College and Research Institute on cases of death due to poisoning. Out of 110 cases, maximum number of cases were male (63.63%), in the age group of 21-30 years (33.63%). 92.72% belonged to Hindu religion and 55.45% belonged to the upper lower class. 54.54% belonged to urban areas and 60% were married victims. Maximum numbers of cases (38.18%) were recorded in winter, commonly between 5pm – 10pm (39.09%) and 84.54% occurred at the place of residence. 84.54% of cases were suicidal in nature with psychological causes being the most common motive (30.90%). 81.81% of cases received treatment and 34.53% succumbed to death within 1-7 days of consumption of poison. Organophosphorus group of insecticide (50%) was the most common poison encountered, followed by Phoshide ions (30%).

Conclusion: Poisoning is a major epidemic of non-communicable disease in the present century. Suicidal and accidental poisoning are significant contributors to morbidity and mortality throughout the world.

Keywords: Demographic profile, Phoshide, Poisoning, Organophosphorus

Introduction

According to the World Health Organization, a poison is a substance (solid, liquid or gaseous), which if introduced to the living body or brought into contact with any part thereof, will produce ill health or death, by its constitutional or local effect or both. As per WHO, about 3 million cases of poisoning with 2,20,000 death occur annually worldwide, of which 90% of the cases occur in developing countries particularly among agricultural workers. According to NCRB (National crime records bureau) statistics of 2015, 37232 persons committed suicide by poisoning in India, of which 23930 persons committed suicide by consuming insecticides and 13302 persons consumed other poisons. 39.1% of deaths (26173) were attributed to accidental poisoning. Poisoning suggests an acute event demanding immediate care and attention.

History indicates that the use of poisons has been known since time immemorial. In the antiquity, the main way to poison an enemy was by adding plant or animal toxins into food and drinks. In the Middle Ages, the progress within this area moved forward. Inorganic toxic compounds prevailed. Moreover, such substances were applied more sophisticatedly, i.e., by poisoning of...
the gloves, page corners, wigs, etc. Additionally, there was one more intermingling feature—usage of poisons for unfair purposes had not been usually proven. Thus, criminals had a big chance to escape the punishment. In the Middle Ages, poisons were freely sold in pharmacies. The first attempt to stop free trade with poisons was made in Italy. In modern day India, there are a number of legislatures in place, such as Drugs and Cosmetics Act of 1940, Narcotics Drugs and Psychotropic Substances Act of 1985 and its 2014 amendment that prevent the illegal manufacture, possession and distribution of poisons and other toxic substances.

Poisoning is a major epidemic of non-communicable disease in the present century. Among the unnatural deaths, deaths due to poisoning come next only to road traffic accident deaths. The choice of poisoning agents depends on availability, cost, harmful effects of poison and regional consideration. Pesticides which were invented to protect crops from rodents, insects and humans from starvation have themselves become an important contributor to unnatural deaths. This study was conducted to know the various influencing factors associated with death due to poisoning like age, sex, marital status, occupation, availability of poison and socio-economic status. Knowledge regarding the type of poison chosen might help in imposing restrictions on the sale of those poisons and hence help in preventing deaths due to poisoning.

**Material and Methods**

All cases of deaths due to poisoning brought to Department of Forensic Medicine and Toxicology, Victoria Hospital, Bangalore Medical College and Research Institute for autopsies between October 2016 to May 2018 were selected on a purposive sampling basis.

Following inclusion criteria were taken into consideration:

1. Autopsy on all cases of deaths due to poisoning conducted at Department of Forensic Medicine and Toxicology, Bangalore Medical College and Research Institute during the study period.

2. Cases confirmed as poisoning by chemical analysis report from Forensic Science Laboratory and based on the hospital case sheet records.

Following exclusion criteria were taken into consideration:

1. Autopsy on unidentified bodies.
2. Autopsy on decomposed bodies.
3. Cases of poisoning where the Forensic Science Laboratory report and/or hospital case sheet records are unavailable.

The details of family history, previous medical history and any treatment records, when available, were obtained from concerned police. Autopsy was performed using the Letulle’s evisceration technique, examining all organs. Viscera and blood were sent for chemical analysis to Forensic Science Laboratory. The data obtained from this study was then analysed statistically using Statistical Package for the Social Sciences (SPSS 20) and the data was presented in the form of appropriate tables, computing descriptive statistics such as percentages.

**Results and Discussion**

During the study period, a total of 110 cases of poisoning were studied and the corresponding chemical analysis reports from Forensic Science were obtained. In this study, the maximum number of cases i.e. 70 were male (63.63%) and the rest i.e. 40 were female (36.36%). This can be attributed to the fact that males form the majority of the population and being the sole breadwinner in most families, have to shoulder many responsibilities making them more vulnerable. Whereas, women who are commonly subjected to post-marital problems such as cruelty by husband/in-laws tend to fall prey to suicides by poisoning. Similar findings were observed in the study conducted by Kanchan T, Menezes RG. In this study, the maximum number of cases i.e. 37 were in the age group of 21-30 years (33.63%), followed by 19 cases in the age group of 31-40 years (17.27%). The least number of cases were observed in the 71-80 years (0.90%) and 81-90 years (0.90%) age group (Table 1). Ambition, adventurous mind, aggressive personality, opportunity hunting, academic pressure and challenges,
allure of opposite sex affect the younger age group. Study conducted by Singh B, Unnikrishnan B noted similar findings, whereas contrasting findings were noted in the study conducted by Lee WJ, Cha ES, Park ES et. Al.

In this study, maximum number of cases i.e. 102 belonged to Hindu religion (92.72%), followed by Muslims (5.45%) and Christians (1.81%). This can be explained by the fact that Hindus form the majority of the Indian population. This is supported by the study conducted by Patel NS, Srivastava AK, Amit Kumar et.al., and the study conducted by Gupta BD, Vaghela PC.

According to modified Kuppuswamy socioeconomic status classification, in this study, it was found the maximum number victims belonged to the upper lower class (55.45%) and the least number of victims (1.81%) in the upper class (Table 2). Lack of education, inability to achieve desired educational qualification, financial inadequacy to pursue education, work pressure, job dissatisfaction, failure to achieve targets, strain in employer-employee relationship, excessive debt, low income, over expenditure are the common issues encountered among the lower strata of the society. Patel DJ, Tekade PR have observed similar findings in their respective studies. Whereas, opposing findings were noted in the study by Bharath K Guntheti, Uday Pal Singh.

The distribution of domicile pattern of the victims showed that 60 were from urban areas (54.54%) and 50 were from rural areas (45.45%). This can be attributed to the fact that this study was conducted in the metropolitan city of Bengaluru. Problems of urbanization include over population, intense competition, adaptability issues, unemployment, stress, high cost of living, financial mishaps, desire to make a quick buck and living beyond one’s means. Contrasting findings were observed in the study by Gargi J, Tejpal H R, Ashok Chanana et. al., and the study conducted by Sharma BR, Nidhi Relhan, Neha Gupta et. Al.

It was noted that the maximum number of cases were seen among married victims i.e. 66 cases amounting to 60%, followed by unmarried victims i.e. 44 cases amounting to 40%. Early marriage, lack of compatibility, high expectations from the spouse, adjustment problems with in-laws and the new environment especially among women, lack of parental support - more so in love marriages, dowry demand, infidelity, domestic violence, cross-cultural clashes are the common issues that plague the institution of marriage. Similar findings were noted in the study by Dipayan Deb Barman, Vijaya Kumar Nair, Karnaboopathy GR. Opposing findings were noted in the study by Patel NS, Srivastava AK, Amit Kumar et.al.

Maximum numbers of cases i.e. 42 were recorded in winter (38.18%), followed by 26 victims each in summer (23.63%) and monsoon (23.63%). Even though it is very difficult to establish a genuine correlation for this, factors such gloomy weather, predisposition to respiratory illnesses, lack of job opportunities could play a significant role. In the study by Tüfekçi I B, Curgunlu A, Sirin F, same observations were made. This finding is deviant from the observations in the study conducted by Kar SM, Sidartha Timsinha, Prashant Agrawal.

It was observed that the most common time of incident was between 5pm – 10 pm (43 cases – 39.09%), followed by 10am – 5pm (35 cases - 31.81%). The least number of cases were observed between 6am – 10am (13.63%). It is not possible to arrive at a precise conclusion for increased number of deaths for the above said duration. However, leisure time after work hours resulting in family interaction and conflicts, recapping of distressing events, alcohol consumption etc. can lead to the fatal event. Similar finding in males was noted in the study by Kanchan T, Menezes RG. Contrasting findings were noted in the study by Bharath K Guntheti, Uday Pal Singh.

The place of residence was the most common place of occurrence of incident as seen in 93 cases i.e. 84.54%. Two cases of accidental cyanide poisoning in children were noted at their father’s workplace which was a goldsmith’s shop (Table 3). Privacy of one’s own home, closed doors and an unsuspicious environment made the victim’s residence the place of choice for such an extreme step. Dipayan Deb Barman, Vijaya Kumar Nair, Karnaboopathy GR noted similar findings in their respective studies.

The present study shows that 84.54% of cases i.e. 93 were suicidal in nature. Accidental poisoning constituted 12.72% of cases and 2.72% of cases were homicidal in nature. Poisoning is one of easiest methods...
of committing suicide. Easy availability, vast spectrum of poisons, cost-effectiveness and surety of death make poisoning an appealing means of committing suicide. In their study, Lee WJ, Cha ES, Park ES et. Al\(^9\) have noted similar findings. The observations in the study by Lan Zhou, Liang Liu, Lin Chang et. al.\(^{18}\) was deviant from these findings.

The most common motive for death was found to be psychological reasons in 34 victims i.e. 30.90% of cases, followed by domestic reasons in 29 victims i.e. 26.36% of cases (Table 4). In the current scenario of rapid and irrational urbanization where ever-changing pattern of life is affecting the individual at all levels – personal, educational, occupational, marital, social, cultural and financial; conflict within oneself and with one’s environment is inevitable. The burden of imbalance of one or more of these factors is borne by the psyche of the victim, leading to the ultimate decision of suicide. Depression, psychosis, bipolar disorder, schizophrenia, drug and alcohol dependence are some of the psychiatric disorders affecting such victims. Domestic reasons include premarital factors such as romantic misendeavours, loss of dear ones, coercion/blackmailing by a trusted partner, conflict with parents and partner rejection; and post-marital factors include lack of compatibility, infidelity, adjustment problems with in-laws, dowry demand, domestic violence etc. Such findings were also observed by Patel NS, Srivastava AK, Amit Kumar et.al.\(^{10}\), in their study.

It was observed that maximum number of cases i.e. 99 were booked under Section 174 CrPC (90%) This can be explained by the fact that majority of the medicolegal autopsies fall under Section 174 CrPC which deals with unnatural deaths. Two cases (1.81%) were registered under Section 174 ‘C’ CrPC which deals with suspicious deaths. Three cases (2.72%) were booked under Section 176 CrPC which deals with Magistrate inquest, conducted when death occurs in police custody. Three cases (2.72%) were booked under Section 306 IPC which deals with abetment of suicide. These were cases of married women where their husband / in-laws were held responsible for the abetment of the victim’s suicide. Two cases (1.81%) were booked under Section 498(A) IPC which deals with husband or relatives of husband subjecting a woman to cruelty. One case (0.90%) was registered under Section 304(B) IPC which refers to dowry death. There were two cases (1.81%) booked under Section 302 IPC which is the punishment for murder. The circumstance of this case was that the mother, who was a victim of domestic abuse, decided to end the lives of her children in a bid to free them from the abuse.

Out of the total 110 cases that were studied, 90 victims were admitted in a hospital and received treatment (81.81%). The rest did not receive any treatment. This study was conducted at Victoria Hospital which is a tertiary care hospital catering to a significant local population and also to the surrounding towns, cities and states; a large majority of patients seek treatment here resulting in good numbers of treated patients. Timely action by the relatives, speedy transport and swift intervention by the doctors also contributed towards majority of victims being treated. High dose of consumption, rapid action of poison and act of poisoning going unnoticed by the relatives could be factors which resulted in non-treatment.

The most common poison encountered in this study was Organophosphorus group of insecticide (55 cases - 50%), followed by Phosphide ions in 33 cases - 30% (Tables 5 and 6). The findings are in accordance with the findings observed in the study by Shetty AK, Jirli PS, Bastia BK\(^{19}\). Opposing findings were noted in the study by Shadnia S, Esmaily H, Sasanian G et.al\(^{20}\), where the most common agent was drugs. In the study by Lan Zhou, Liang Liu, Lin Chang et. al.\(^{18}\), the most common poison was the rodenticide tetramine and in the study by Sharma BR, Nidhi Relhan, Neha Gupta et.al.\(^{15}\), the most common poison was aluminium phosphide. Easy availability over the counter as well as an accessible poison in the form of insecticides especially in the house of farmers, cost effectiveness, fatal nature of the compound, awareness about toxicity of compound among general population make organophosphorus compound the most common poison encountered. Its effectiveness as a rodenticide, regular use in grain storage, unsafe storage practices and easy accessibility as a household poison especially among women, makes Phosphide compounds second in popularity.
It was observed that maximum number of victims (34.53%) succumbed to death within 1-7 days of consumption of poison, followed by 17 cases (15.45%) were brought dead. Only 6.36% of cases survived for less than 1 hour. This is in contrast to the findings noted in the study by Shetty AK, Jirli PS, Bastia BK.

The intermediate clinical manifestations observed in Organophosphorus compound consumption could be responsible for the delayed deaths. In addition, the effective therapeutic measures would have prolonged the toxic sequelae of Organophosphorus compounds resulting in death at a later date. Majority of the time, the victims ingest formulated Organophosphorus compounds instead of pure active ingredients. Different compounds such as xylene or cyclohexanone maybe be used as solvents. The clinical manifestations of poisoning with these solvents are often poorly studied and uncertain. Other individual-specific factors like general condition of the patient, tolerance, delayed gastric emptying time, response to treatment could have also contributed to the delayed deaths. In our study, it was observed that 10 cases of Phosphide poisoning succumbed to death in 1-7 days. Even though phosphides cause immediate deaths due to rapid toxicity, the delayed deaths in our study may be due to low dosage, reduction in potency due to interference with atmospheric moisture and effective management of dysrhythmias which may otherwise lead to immediate death.

**Conclusion**

Public education programs about suicide are an important component of pesticide-suicide prevention programs. They suggest that public education should focus on convincing the public that suicide is an important public health problem that can be prevented. Educational activities should aim at changing the prevailing attitudes. Significant number of deaths can be prevented by providing local first-aid kits, better training of physicians, faster transportation to hospitals, and ensuring that adequate supplies of antidotes and essential hospital equipment are readily available. Poison information centres are uniquely centralized repositories of data about human exposures to chemicals, including information about the agents involved, the circumstances giving rise to exposure, and the health effects of exposure. Establishment of such centres help reduce the incidence of poisoning by identifying emerging toxicological hazards, stimulating preventive measures by manufacturers and regulators; and assessing the efficacy of such measures.

**Ethical Clearance:** A prior approval was obtained from the Institutional Ethics Committee, BMCRI.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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1. WHO definition of poison [Internet]. Available from: http://www.who.int/topics/poison accessed on 24/10/2018.


Assess the Effectiveness of “First Aid Training Program” in Terms of Knowledge and Skills Among School Teachers of Selected Schools of Ambala, Haryana: A Quasi Experimental Study

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Abstract

Background: As the incidence of medical emergencies is rising, the demand of the knowledgeable and skilled personnel is also needed. It is important for every person to be competent enough to tackle all the emergency situations anywhere. First aid training helps to make a person able to give actual immediate care and safety awareness. The objective of the study is to assess the effectiveness of first aid training program in term of knowledge and skill among school teachers.

Methodology: A Quasi-Experimental study following one pre-test post-test design was used. Participant’s data was collected from 105 school teachers from 12 schools (9 govt. and 3 private) using Total enumerative sampling and the schools were conveniently selected. Knowledge and skills were assessed by using structured knowledge questionnaire and observational checklist respectively.

Conclusion: Data was analyzed using SPSS 16. The t test analysis showed a significant difference before and after the administration of first aid training program in term of knowledge(t=37.05 and p= 0.001) and skills (t=84.485 and p=0.000) at 0.05 level of significance. There was significant association of pre-test knowledge scores with educational status of school teachers.

Key words: effectiveness, First Aid Training Program, knowledge, skills, school teachers.

Introduction

First aid is a valuable key for all beings. It helps individual to become efficient in providing immediate care to the on spot victim, also it helps him to assist persons who are willing to help other injured ones. The knowledge and practice of First Aid is important in limiting the pain and complications brought out by negligence and it can improve the condition at hand only, more the comprehensive knowledge and efficient skills, less will be the risk of mortality.1

The First Aider should always be prepared in order to save life.2 The common types of school accidents are fainting, epistaxis, shortness of breathing, fractures, joint dislocation, bruises, burns, choking, seizures, insect bites and poisoning.3
According to the study by Henry et al at America found that 30% of children younger than 5 years and 56% of children, aged 6 to 10 years had at least one episode of nosebleed. According to the report by Heart institute at Cincinnati’s children’s pediatric heart program, around 20% of young children’s parents reported their children underwent fainting.

According to Indian guidelines and protocols it has been mentioned that honey bee sting and insect sting accounts for 1% incidence in children and 3% incidence in adults. A bee sting is always potentially dangerous as 30-50 stings from a bee have been proved fatal.

Teachers are the main caregivers and the first line of protection for school students. Their role complements that of parents. They are the first respondent in cases of disasters or emergencies. Therefore, they must be able to deal appropriately with medical emergencies, for all healthy students and those students with special health care needs. This role can be achieved if teachers are equipped with the required skills to provide effective First Aid services that promote recovery and prevent future serious health consequences.

Material and Method

The study was conducted during the period from March 2018 to June 2019 in the state of Haryana, India as a Quasi-Experimental research design “one group pre-test post-test design”. The ethical clearance was obtained from university research ethics committee (MMU/IEC/1305) and the study was carried out in accordance with the guidelines laid by Indian Council of Medical Research ICMR (2006). The written consent from school teachers was collected prior to the study. A sample of 105 school teachers from 12 schools (09 from Government and 03 from private school) participated in this study with the prior permission from the principals of selected schools and board of education Mullana. The sample was selected through Total Enumeration Sampling Technique. The sample size for the study was estimated using Cochran’s sample size formula. First aid training program was given with Lecture cum discussion and demonstration method with the help of power-point presentation, videos and demonstration in simulated setting using case scenarios regarding First Aid Management of selected conditions including wound care, fracture, epistaxis, choking, fainting and honey bee sting.

DESCRIPTION OF TOOL

Section I: description of sample characteristics

It consisted of nine items related to selected sample characteristics of school teachers i.e age, Gender, education status, job experience, marital status, no of children, previous Knowledge of First Aid, if yes then source of information, previous handling of First Aid, if yes, then specify condition, previously had taken any First Aid Training.

Section II: Structured knowledge questionnaire

A preliminary list of 30 items on knowledge was prepared. All the 30 items were multiple choice type questions. Each item had a single correct answer. Every correct answer was accorded a score of one mark and every wrong answer assigned zero mark. Thus the maximum score was 30 and the minimum score was zero. The level of knowledge was categorized as very good, good, average and below average.

Section III: An observational checklist

For skill assessment of school teachers on selected condition of First Aid. Paper pencil technique was used for description of selected sample characteristics and structured knowledge questionnaire. Observation technique was used to assess the skills of school teachers.

A preliminary list of 64 items on observational check list was prepared on management of wound care, fracture, epistaxis, choking, fainting and honey bee sting. Each item was marked as yes if followed or no if not followed. Total score ranges from 0-64 and the maximum possible score was 64 and minimum possible score was zero. The level of skills was categorized as very good, good, average and below average.

The reliability coefficient for structured knowledge questionnaire was calculated by using Kuder-Richardson- 20 (KR20) formula and it was found to be 0.8.

Data Analysis

KS (Kolomogorov- Smirnov) test was applied to check the normality of the data. Data was normally
distributed hence parametric tests were applied. The entire hypothesis was tested at 0.05 level of significance.

**Descriptive statistics**

- Frequency and Percentage Distribution to describe sample characteristics.

- Frequency and percentage distribution, Range of score, Mean, Median and Standard Deviation

**Inferential statistics**

- Karl pearson correlation was used to find relationship between knowledge and skills

- ANOVA and t-test was used for association of selected sample characteristics with knowledge and skill among school teachers.

**Result**

Data was entered in Microsoft excel and analysis was done with SPSS version 16. Result of the study shows that first aid training program is effective in improving the knowledge and skills among schoolteachers.

1. **Findings related to selected sample characteristics:**

In the present study, Less than half of the subjects (32.2%) were above 40 years. Almost all (98.09%) of them were female, more than (54.28%) of them were post graduate and less than half (34%) of the school teachers had 6-10 years of experience. Majority (92.28%) of them were married, for less than half (39.47%) of them source was friend and relatives and More than half of teachers did not 53 (50.47%) performed first aid previously.

2. **Findings related to the effectiveness of First Aid Training program in terms of knowledge among school teachers**

In pretest of knowledge, majority of the school teachers (85.71%) had below average and only few (14.28%) had average knowledge whereas in post test more than majority of the teachers (80%) had good level of knowledge , few (10.47%) had very good level of knowledge and less than few (9.52%) had average level of knowledge.

**TABLE 1: Mean, Mean Difference, Standard Deviation of Difference, Standard Error of Mean Difference  
‘t’ and ‘p’ value of Pretest and Posttest of Knowledge Score of School Teachers**

<table>
<thead>
<tr>
<th>Knowledge Score</th>
<th>Mean</th>
<th>M D</th>
<th>SDD</th>
<th>SEMD</th>
<th>‘t’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Test</td>
<td>11.59</td>
<td>11.524</td>
<td>3.187</td>
<td>0.311</td>
<td>37.054</td>
<td>0.001**</td>
</tr>
<tr>
<td>Post-test</td>
<td>23.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“t”(104) = 1.660 at 0.05 level of significance

**Table 1 shows** that the level of knowledge among school teachers after administration of first aid training program. The t test was applied and the mean difference was found to be statistically significant (“t”=37.054, p=0.001) at 0.05 level of significance. Hence, it can be inferred that the First Aid Training Program was effective in increasing the knowledge of school teachers on First Aid Management.

3. **Findings related to the effectiveness of First Aid Training program in terms of skills among school teachers**

In pre-test of skills through observational checklist all (100%) of the school teachers were having below average skills. Whereas in the post-test most of the school teachers (73.33%) were having good skills, 24.76% were having very good skills, 0.009 were having average skills and 0.009 below average skill.
Table 2
Mean, Mean Difference, Standard Deviation of Difference, Standard Error of Mean Difference ‘t’ and ‘p’ value of Pretest and Posttest of Skills Score of School Teachers

<table>
<thead>
<tr>
<th>Skills score</th>
<th>Mean</th>
<th>MD</th>
<th>SDD</th>
<th>SEMD</th>
<th>‘t’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>4.21</td>
<td>49.59</td>
<td>45.381</td>
<td>5.504</td>
<td>84.485</td>
<td>0.000**</td>
</tr>
<tr>
<td>Post-test</td>
<td>4.95</td>
<td>49.59</td>
<td>5.048</td>
<td>0.537</td>
<td>84.485</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

“t” (104)= 1.660 at 0.05 level of significance
**highly significant p<=0.001

Table 2 depicts that the level of skills among school teachers after administration of first aid training program. The t test was applied and the mean difference was found to be statistically significant (“t”= 84.485, p=0.000) at 0.05 level of significance. Hence, it can be inferred that the First Aid Training Program was effective in increasing the skills of school teachers on First Aid Management.

4. Findings related to the relationship between knowledge and skills among school teachers on selected conditions of First Aid

TABLE 3: Correlation between Knowledge and Skills Scores

<table>
<thead>
<tr>
<th>Knowledge</th>
<th>Pre test</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skills</td>
<td>-0.06(0.95)</td>
<td>-0.04(0.6)</td>
</tr>
</tbody>
</table>

df(104 ),r(105)= 0.194 significant(p<=0.05)

Table 3 depicts the relationship between knowledge and skills of school teachers regarding First Aid Management of selected conditions. During Pre-test no significant relationship was observed between knowledge and skills(r=-0.06,p=0.95). During Post-test also, no significant relationship was observed between knowledge and skills(r=-0.04,p=0.6). The calculated r-value in pretest and post-test shows that there was no significant relationship between Knowledge and skills. This indicates that there is no significant relationship between knowledge and skills score of school teachers on First Aid Management of selected conditions.

5(A) Findings regarding association of knowledge scores of school teachers regarding First Aid with their selected sample characteristics using t-test and ANOVA

The ANOVA/’t’ value of age (0.729), gender (1.326), job experience (1.64), marital status (0.54), no of children (1.090), Previous knowledge regarding First Aid management (0.48), Source of information (3.230), Have you performed first aid previously (0.41) were found to be statistically non-significant except educational status (8.442), hence post hoc test was applied. It infers that knowledge was independent of selected sample characteristics except educational status (Post Graduates had higher knowledge than J.BTs).
Table 4: Post hoc Test Showing Mean Difference for Association of Pre Knowledge Scores with Selected Sample Characteristics

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample Characteristics</th>
<th>Categories</th>
<th>Mean Difference</th>
<th>Standard Error</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Educational status</td>
<td>J.BT vs. B.Ed.</td>
<td>0.965</td>
<td>0.971</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J.BT vs. Postgraduate</td>
<td>3.341</td>
<td>0.950</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B.Ed. vs. J.BT</td>
<td>1.965</td>
<td>0.971</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B.Ed. vs. Post graduate</td>
<td>1.376</td>
<td>0.486</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post graduate vs. J.BT</td>
<td>3.341</td>
<td>0.950</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post graduate vs. B.Ed.</td>
<td>1.376</td>
<td>0.486</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Table 4 shows significant mean difference for association of knowledge with educational status. It depicts that school teacher who has the education status as Post Graduation had better knowledge than the teachers with education status as JBT (Junior Basic Training) as mean difference was significant at 0.05 level of significance.

5(B) Findings regarding association of skills scores of school teachers regarding First Aid with their selected sample characteristics using t-test and ANOVA

The ANOVA/‘t’ value of age (0.869), gender(0.461), educational status(2.648), job experience (1.683), marital status(0.72),no of children(1.037), Previous knowledge regarding First Aid management (0.326), Source of information(0.455), Have you performed first aid previously (0.185) were found to be statistically non-significant. It denotes no association with knowledge.

Discussion

The main aim of the study was to evaluate the effectiveness of First Aid Training Program in terms of Knowledge and skills of school teachers related to First Aid of selected conditions.

Effectiveness of First Aid Training Program in terms of Knowledge among school teachers related to First aid of selected conditions.

In the present study the mean post-test knowledge score of school teachers (23.911+1.923) was higher than the mean pretest knowledge score(11.15+ 2.5378). The computed “t” value (37.054) was found to be significant at 0.05 level of significance. Hence, the null hypothesis ($H_0$) was rejected and research hypothesis ($H_1$) was accepted. This indicates improvement in knowledge after the administration of First Aid Training Program on first aid management of common conditions. These findings were consistent with a study conducted by Sumithram 2017 where mean post-test knowledge score of school teachers (25.3+3.15) was higher than the mean pretest knowledge score(18.9+ 2.99).

Effectiveness of First Aid Training Program in terms of Skills among school teachers related to First aid management of selected conditions.

In the present study, there was a significant difference in the mean skill score. The post-test skill score (49.59+4.632) was higher than the mean pre-test skill score (4.21+2.191). The computed “t” value (84.48) was found to be statistically significant at 0.05
level of significance. Hence, the null hypothesis ($H_{02}$) was rejected and research hypothesis ($H_2$) was accepted. Thus, it can be inferred that the First Aid Training Program was effective in improving the skills of school teachers related to First Aid of selected conditions. These findings were consistent with a study conducted by Shobha Masih2017 where there was a significant difference in the mean post-test skills score (18.52±2.63) was higher than the mean pre-test skill score (14.52±2.39).

**Conclusion**

The first aid training program was effective in enhancing knowledge (pretest score=11.15±2.5378 and post test score=23.911±1.923) and skills (pre-test 4.21±2.191 and post-test 49.59±4.632) of school teachers on common conditions of first aid. There was no relationship between knowledge and skill scores of schoolteachers on common conditions of first aid. There was no significant association of First Aid Training Program with pre-test knowledge and Skills scores.

**Conflict of Interest:** NIL

**Funding Sources:** NIL

**Ethical approval:** The ethical clearance was obtained from university research ethics committee of Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala (MMU/IEC/1305) and the study was carried out in accordance with the guidelines laid by Indian Council of Medical Research ICMR. The permission was taken to conduct the study from the principals of the selected institutes. The written consent from the students was collected prior to the study. The purpose for carrying out research project was explained and assurance of confidentiality was given to the participants.

**References**


Oral Hygiene as a Risk Factor in Periodontitis

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Abstract

Dental plaque is the etiological factor for initiation of periodontal disease due to secretion of toxins and proteolytic enzymes by the microbial accumulation in the cervical region of teeth & sub gingival area.

Gingival inflammation will progress into the continuing destruction of supporting tissues like resorption of alveolar bone Joss of attachment increase pocket depth if we don’t remove the microbial dental plaque, by daily oral hygiene practice through mechanical tooth brushing of the person himself & periodic clinical examination to remove the plaque, calculus & other retentive factors to maintain the oral hygiene in a good manner.

The oral hygiene is considered as a risk factor in the prevention or progression of periodontal disease depending on daily removing of dental plaque by the person & professional recalling system clinically.

Key Words: Gingival inflammation, Dental plaque, patient, Iraq

Introduction

The aim of personal oral hygiene combined with professional cleaning by clinical parameters is to remove the dental microbial plaque. (1,2) The oral hygiene done by the subject include the mechanical tooth brushing, dental flossing, Interdental brushing & chlorhexidine mouth wash 0.2% combined with deep scaling, subgingival duration of bacterial groups, including the roots of infected teeth & polishing which done by the dentist clinically through periodic table once in month of at least. (3,4,5,6)

By these previous two steps we can maintain the oral hygiene in a good manner & prevention of the disease with treatment of periodontal lesion which include treatment of gingivitis, new attachment Level gain, decrease pocket depth, decease tooth mobility & formation of new bone support of teeth. (8,9,10)

Even the periodontitis done by surgical interference, there should be a post-surgical maintain of oral hygiene by the patient combine with clinical recalling system by the operator to maintain The oral hygiene by motivation & give instruction to the patient about the oral hygiene by himself & periodic visit to the clinic scaling in order to remove the accumulation of dental plaque continuously. (11)

This issue bears some limitations. In addition, previous studies have reported inconsistent findings in this regard. With this background in mind, the present study aimed to compare infertility-related stress among the infertile couples and its relationship with infertility factors.

Materials and Methods

1- Selection of 20 patients complaining from periodontitis.

2- Divided in 2 groups: test group & control group.

3- on base line. we record the following clinical parameters of the 2 groups.

a- Collection of dental plaque sample sub gingivally to identify the presence of microorganisms by an immunological assay method which as follow:

This method uses for detection of sub-gingival pathogens (antigen) using antibodies specific to that bacterial antigens. collection of plaque samples of sterile paper points (diluted) fixed to slide
(stained by incubation with antibodies)

this technique demonstrated that the presence of specific pathogens corresponded with increasing pocket depth.

this test is passed on antigen-antibody binding in the subgingival dental plaque.

(b) At base line: -

1- Report the personal and hygiene practice.

2- Measurement of clinical periodontal examination by periodontal probe for each subject that include: plaque index, probing pocket depth, attachment level.

(c) data collection is obtained for each subject clinically for the presence of microorganism subgingivally & the oral hygiene practice (at baseline) & then compare it with the results at the end of this study in the healing of periodontitis after following of good oral hygiene measurement of self-perform plaque control practice & by clinical recall system including the (control group) only.

But the (test group) which are not include this system of oral hygiene (personal & clinical) but they follow only the person method of hygiene without clinical follow up system and compare them with a control group.

Results

All subjects at baseline are subjected to plaque index (40%), measurement pocket depth (5mm), and the attachment level (1.34mm)

In test group: -

1- Plaque index: after 6 months was 35% Plaque index; after (1) year of the trial was 30%.

2- Pocket depth: at the beginning of the trial was 5mm Pocket depth at the end of the trial was 3.6mm.

3- The attachment level at first was 1.34mm

Control group

<table>
<thead>
<tr>
<th></th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>6month</th>
<th>End of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index%</td>
<td>40%</td>
<td>30%</td>
<td>22%</td>
<td>14%</td>
<td>5%</td>
</tr>
<tr>
<td>Pocket depth (mm)</td>
<td>4.8mm</td>
<td>4.2mm</td>
<td>3.6mm</td>
<td>2.9mm</td>
<td>2mm</td>
</tr>
<tr>
<td>Loss attachment level (mm)</td>
<td>1.34mm</td>
<td>1.26mm</td>
<td>1.12mm</td>
<td>1mm</td>
<td>0.8mm</td>
</tr>
</tbody>
</table>

Test group

<table>
<thead>
<tr>
<th></th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>6month</th>
<th>End of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index%</td>
<td>40%</td>
<td>38%</td>
<td>36.2%</td>
<td>35%</td>
<td>30%</td>
</tr>
<tr>
<td>Pocket depth (mm)</td>
<td>5mm</td>
<td>4.8mm</td>
<td>4.7mm</td>
<td>4mm</td>
<td>3.6mm</td>
</tr>
<tr>
<td>Loss of attachment level (mm)</td>
<td>1.34mm</td>
<td>1.3mm</td>
<td>1.28mm</td>
<td>1.2mm</td>
<td>1.12mm</td>
</tr>
</tbody>
</table>

The attachment level at the end of trial was 1.12mm

In control group: -1-plaque index:
1st month 40%
2nd month 30%
3rd month 22%
4th month 18%

Continue to reduce the percentage until it reaches 5% at
the end of the trail

2- pocket depth: -
1st month 4.8 mm
2nd month 4.2 mm
3rd month 3.6 mm
4th month 3.1 mm

Continue to reduce the depth of the pocket until it
reaches 2 mm at the end of the study.

3-the attachment level lose: -
1st month: 1.34 mm
2nd month: 1.26 mm
3rd month: 1.12 mm

It continues to reduce the level of losing the attachment
until it reaches 0.8 mm at the end of the study.

Discussion

Despite that, there is a putative pathogen in the
area of shallow subgingival pocket, but the recalling
regular personal clinical oral hygiene status has a value
in reducing the problem of progression of periodontitis.
(12,14) This is a duo to continue removing the bacterial
level of pocket depth. plaque index, attachment level
at baseline & at the end of this study. (13) The risk of
oral hygiene will be reduced by following the periodic
program of removing the accumulation of dental plaque
& examine the self-perform oral hygiene & clinical
checking of the plaque remnants. (15,16)

Conclusion

Regular personal self-perform & clinical
professional dental care are of essential factors to
reduce the problem of periodontitis. This is done
by the regular periodic program for continuing to
remove of bacterial accumulation of dental plaque in
the shallow subgingival pocket depth by healing the
attachment level & reducing the plaque index with the
healing of periodontal problems.

Conflict of Interests:Authors declare that they
have no conflict of interests.

Ethical Issues: Not applicable.

Financial Support: We do not receive any funding
for this project.

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Incidence of Hydatidosis in Slaughtered Animals and their Relation to Public Health at Baghdad Province

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Abstract

Hydatid cysts disease is an important public health problem and economic losses especially at developing countries such, Iraq. A study of incidence of hydatidosis in sheep and cattle, which are slaughtered food animals, was carried out at different areas of Baghdad province outside the government abattoirs, during a period of 18 months from November 2016 to May 2018. Both slaughtered livestock animals (sheep and cattle) which are local breed of both sexes, they are of difference areas in boundary at Baghdad city, and with ages having non descriptive features. Hand palpation and visual inspection were followed in this study. A total, 2594 carcasses were examined comprising 1632 sheep and 962 cattle, from these 33, 11 (sheep and cattle) respectively were found to harbour the cysts, recording percentages of (2.0%, 1.1%) respectively. The predominant location cysts at the liver (48.4%, 45.4%) in sheep and cattle respectively, next by the lungs, in sheep and cattle (39.3%, 36.3%) respectively. Next to the mixed organs (liver + lung), the incidence was (18.1%) in cattle and (9.0%) in sheep. The percentage of cysts in heart of sheep was (3.0%) but in cattle it was (0%) Zero. The results revealed that fertile cysts only, were present in sheep with (87.8%) fertility. Cysts in the organs ranged(1-13,2-7) in sheep and cattle respectively. It can be observed that, sheep play a major role for distributions the disease. Suggestive control measures and public health hazard were mentioned.

Keywords: Hydatidosis; Incidence; Cyst; Abattiors; Fertility; Carcasses

Introduction

Hydatid cysts disease or Hydatidosis is one of the silent helminthic zoonotic infection. The larval stage of cestode belonging to the genus *Echinococcus* (family teaniidae) was the main reason of the disease, which considers major public health problem (1).

Cystic hydatidiosis is of worldwide distribution in the Mediterranean region, including countries of the Middle East (2). The infected stage (Hydatid cyst) is the larval stage of the canine tapeworm found in the internal organs of infected herbivores (3,4). Adult worms produce eggs which is passed with the dogs fecaes (7,8). If eaten by herbivores or humans, the eggs hatch releasing larvae (oncosphere) that invade through, the intestinal wall and evolve to hydatid cysts (9,10).

The four types of medical *E*. and public health importance are; *E. granulosus* (which causes cystic echinococcosis), *E. multilocularis* (which, causes, alveolar echinococcosis), *E. vogeli* and *E. oligarthrus* (cause polycystic echinococcosis) (11,12).

Parasite is common in countries where dogs are close contact with humans, and where favourable conditions of life cycle of the parasite are available, as presence of wide variety of hosts, the lack of proper slaughter facilities (13). The disease infects the livestock food animals, that causes, reducing of their production and high economic losses (14). In Iraq Jarjes and Al-Bakri (15). Said that the infestation rates of food animals species range between zero and 40% as a results of previous studies of local workers in Iraq. The present study was desigen to investigate the prevalence of hydatid cysts disease among slaughtered food animals (sheep and cattle) to provide some necessary required data of public health to prevent or at least to minimize the possible hazards of disease.

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Materials And Methods

In this work a survey was carried out to determine the prevalence of hydatid cysts at different areas of Baghdad province where livestock food animals were slaughtered outside the government abattoirs during a period of (18 months) from November 2016 to May 2018. Regular visits were performed weekly to the places of animals slaughtering in which butchers men selling of meat. Most the animals slaughtered come from surrounding areas of rural region. A total of 2594 carcasses, comprising 1632 sheep and 962 cattle were examined during post-mortem inspection to identify hydatid cysts using perfect examination and search of cysts were done by visual inspection, hand palpation and incition of the organs which are; liver, lung, mixed organs (liver and lung) and heart. The hydatid cysts fluid was collected from individual cyst, washed by phosphate buffer saline and checked for the presence of protoscoleces, by sending the samples to the laboratory by suitable box and do centrifugation for the cysts fluid contents at 3000 RPM for 5 minutes, then microscopically examined for the detection of protoscoleces (Latif, et al (16). The cysts were recorded according to animal species, total number of examined animals, the number of animals harbouring hydatid cysts and the cyst location in the organs.

Results

The infection rates of cysts for both species that were slaughtered in different areas of Baghdad province was shown in table (1).

A total of 2594 carcasses of slaughtered food animals were examined, comprising (1632) sheep and (962) cattle, of these numbers (33) sheep and (11) cattle were found to have the cysts, recording percentages (2%,1.1%) respectively. Table.(2) Showed the distribution of hydatid cysts in different organs; which revealed that, in sheep and cattle, liver was more commonly infected with hydatid cysts than the lungs and other organs, recording incidence of (48.4%, 45.4% respectively). In case of lung, in sheep and cattle the incidence (39.3%, 36.3%) respectively.However in mixed organs (liver and lung) the higher incidence was (18.1%) in cattle and lower in sheep (9.0%). The heart was only affected in sheep (3.0%) but in cattle it was none (0%).

The current study revealed that only sheep has fertile cysts (87.8%), while cattle, has sterile cysts these sterile cysts had calcified or caseated appearances. Table. (3) showed that the intensity of cysts was relatively high in sheep (1-13) cyst and less in cattle (2-7).And also the number and fertility percentage of cysts present in sheep and cattle.

Table 1: Number of examined carcasses of slaughtered animals that infected in this study.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>No. Examined carcasses (No.)</th>
<th>No. Infected Carcasses (No.)</th>
<th>Percentage Of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>1632</td>
<td>33</td>
<td>2.0 %</td>
</tr>
<tr>
<td>Cattle</td>
<td>962</td>
<td>11</td>
<td>1.1 %</td>
</tr>
<tr>
<td>Total</td>
<td>2594</td>
<td>44</td>
<td>1.6 %</td>
</tr>
</tbody>
</table>

Table (2): Distribution of hydatid cysts at infected organs of infected carcasses.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>No. of Infected carcasses</th>
<th>Infected organs</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Lung</td>
<td>Liver+lung</td>
<td>Heart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Sheep</td>
<td>33</td>
<td>16</td>
<td>48.4</td>
<td>13</td>
<td>39.3</td>
</tr>
<tr>
<td>Cattle</td>
<td>11</td>
<td>5</td>
<td>45.4</td>
<td>4</td>
<td>36.3</td>
</tr>
</tbody>
</table>
Table (3): incidence of fertile, sterile and intensity of hydatid cysts in contaminated organs.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>No. of Infected</th>
<th>Cysts status</th>
<th>Fertility (%)</th>
<th>Intensity of cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fertile</td>
<td>Sterile</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>33</td>
<td>29</td>
<td>4</td>
<td>87.8</td>
</tr>
<tr>
<td>Cattle</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Hydatidosis is more important and wide spread infection in the world. livestock species are more susceptible to infection by contamination through the viable eggs of Echinococcus granulosus (18). Human risk for the cysts is by ingestion food or water contaminated with fecal material containing E.granulosus eggs passed from infected dogs (19,20). In most studies dealing with the incidence of hydatidiosis in livestock, the main source of data is obtained from abattoirs (21). In this study the data was obtained from different areas at Baghdad city or province where slaughtering animals carried out at the places outside the abattoirs, with absent of the veterinarians supervision. Cystic hydatidiosis was recorded early in Iraq by Babero et al (22).

Adult worm i.e E. granulosus was reported earlier in the 1940 in the intestine of stray dogs (23). As regards to the infection rate of hydatid cysts, the current study revealed that, the lower rates of infection in an examined slaughtered animals were relatively similar to the results of study conducted by Iraqi researchers (15) in Mosul city, (24) in Kirkuk.

The lower incidence in sheep was attributed to the nature of grazing that may be done Torgerson and Buke (25) but the lower incidence of infestation in cattle, is due to breed difference, no contact with source of infection (26). Results of infection rates of this study were lower than those studies by a number of researchers in Iraq (27-32).

And also studies that were reported in some Arabian and neighbouring countries i.e Egypt (13), Jordan (33), Kuwait (34), Yemen (35), Iran (14) and Turkey (36). The main feature of the present work, is the small sample of animals slaughtered compared with previous local studies (27-32). The variation of infection rates could be related to differences among strains of E. granulosus (21).

Regarding to infection rates of organs involvement, the commonest sites of infection are, the liver and lung Markell et al (37). Liver in sheep and cattle is the predominant location of the cysts followed by lung, mixed organs (liver+lung) and heart later. These results are relatively similar to that findings by Jarjes and AL-Bakri (15). Muqbil et al (35), in Aden, Yemen and Elmajdoub and Rahman (21), in Libya. The present study showed that, only sheep were found to harbour fertile cysts, while in cattle all the cysts were sterile.

All local studies mentioned above confirm that, fertility rate of cysts in sheep is higher than other livestock animals, so it is in agreement to the results of this study.

Variation in fertility rate among different intermediate hosts was, due to the difference in E. granulosns strains (39). Sterile cysts of cattle in current study were similer to those studies by Al-Abassy et al (28) . In Iraq , Elmajdoub and Rahman (21), in Libya. The life cycle for parasite, disseminating the disease due to high infection rates and fertility. The recommended measures which are suggested to prevent or at least to minimize hydatidosis disease are. Rehabilitation and improve Baghdad abattoirs and abattoirs of the all provinces in Iraq, prevent slaughtering livestock animals outside the government abattoirs, make control programs for Standard meat inspection under supervision of veterinarians, control of dogs by killing stray dogs, vaccination against hydatid cyst disease in
sheep is advisable.

**Ethical Clearance**: Taken from Dijlah University ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

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Lip prints and bloodstains may be left behind in many crime scenes and may reveal the identity of the victim and the suspected person. This study was aimed to investigate the inheritance pattern of lip prints and blood groups among parents and their offspring in the Javanese population. 105 subjects from 25 Javanese family frames participated in this study. Lips print pattern was recorded and analyzed according to Suzuki and Tsuchihashi classification. A blood group test was also performed for each subject. The statistical analysis carried out using IBM® SPSS® Statistics version 23.0 (IBM, Armonk, New York, USA). The results of this study revealed that type II was the predominant pattern of lip prints among the Javanese population (34.3%). Blood group A was the predominant type in subjects with lip prints type II (15%). The result also shows that the lip prints pattern in girls tends to be inherited from the mother. However, the inheritance of lip prints pattern in boys couldn’t be determined precisely. The heritability of lip prints pattern was observed between parents and their offspring. Also, there was a tendency of blood groups to have a certain pattern of lip prints.

Keywords: blood group, forensic odontology, heritability, lip prints, personal identification

Introduction

Establishing the individual identity is a primary importance at the beginning of the criminal cases investigation. According to Interpol DVI Guide, 2018, teeth, DNA, and fingerprints are the primary and the most reliable means in human identification. Besides, several biological evidence such as lip prints, bloodstains, and saliva may support the process of human identification.

Many studies have been developed to explore the usefulness of lip prints pattern in personal identification based on its uniqueness and permanence. Amongst the established methods, the classification by Suzuki and Tsuchihashi is the most commonly used and simple method for lip prints analysis. Suzuki-Tsuchihashi classified the lip prints pattern as: complete straight grooves (type I), half-straight grooves (type I'), branched grooves (type II), intersected grooves (type III), reticulated grooves (type IV), and undifferentiated grooves (type V).

A study by Patel et al, 2010, in Malaysia, involved 20 sampling frames (10 families with siblings and 10 families with twins) reveals that the lip prints pattern was inherited from parents to their offspring in 2 out of 5 families with twins' children. It was also found that most subjects with type II of lip prints tend to have the blood group type A.

In the actual cases, correlating lip prints with blood groups may help the forensic team to establish the human identity accurately. The objective of this study was to investigate the inheritance pattern of lip prints and blood groups among parents and their offspring in the Javanese
The Javanese population is the largest ethnic group in Indonesia who predominantly located in the central and eastern parts of Java Island, Indonesia.

Methods

Data acquisition

This study was conducted under the approval from the Ethical Committee of Faculty of Dental Medicine Universitas Airlangga, Surabaya (number: 302/HRECC. FODM/V/2019). 105 subjects from 2 generations (parents and children) in 25 family frames who met the inclusion criteria participated in this study. The inclusion criteria of this study are:

- Javanese family consisting of father, mother, and one or more biological children.
- Without cross-marriage history in the family.
- Agreed to participate in this study

The exclusion criteria of this study are:

- Subjects with a history of aesthetic surgery on the lips.
- Subjects with hypersensitivity to lipstick.
- Subjects with congenital abnormalities appear on the lips.

All subjects were asked to complete the informed consent after a brief explanation of their participation in this study. All of the individual lip prints and blood group were analyzed.

The subject was asked to set up straight with relaxed position of their lips. The research personnel cleaned up the lip’s surfaces using a wet cotton swab and gently applied one layer of lipstick (Purbasari No. 83, PT Gloria Origita Cosmetica, Indonesia). After 2 minutes, a scotch tape (5 x 12 cm) was gently stacked on the lips surfaces and removed smoothly with a single motion from left to right (Fig. 1). The imprinted lip prints were then pasted on a white paper (Fig. 2) and analyzed using Suzuki and Tsuchihashi classification (Fig. 3). The blood groups (ABO) test was performed for each subject using antigen serum (Glory Widal Serology Test Kit, PT. Medika Farma Alkesindo, Indonesia).

![Figure 1](image.png)

Figure 1 A scotch tape (5 x 12 cm) was gently stacked on the lips surfaces and removed smoothly with a single motion from left to right.
Figure 2 Recorded lip prints pattern obtained from one family frame.

Figure 3 Lip prints classification according to Suzuki and Tsuchihashi, 1971.
Statistical analysis

The classification of lip prints pattern and the blood type were tabulated and analyzed using Spearman and Pearson test to investigate the correlation of its inheritance pattern from parents to their offspring. The Fisher Exact test was used to observe the significance of the tendency of lip print patterns towards the blood type. The statistical analysis of this study was carried out using IBM® SPSS® Statistics version 23.0 (IBM, Armonk, New York, USA).

Results

Table 1 shows the overall appearance of lip prints pattern in all subjects of this study. These results indicate that type II is the most dominant lip prints pattern among the Javanese population, with 36 appearances out of a total 105 subjects (34.3%), while type V was not visible in all subjects.

Table 1 The percentage of lip prints pattern in all subjects (total of 105 subjects)

<table>
<thead>
<tr>
<th>Lip Print Type</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>22 (21.0%)</td>
</tr>
<tr>
<td>Type I’</td>
<td>16 (15.2%)</td>
</tr>
<tr>
<td>Type II</td>
<td>36 (34.3%)</td>
</tr>
<tr>
<td>Type III</td>
<td>17 (16.2%)</td>
</tr>
<tr>
<td>Type IV</td>
<td>14 (13.2%)</td>
</tr>
<tr>
<td>Type V</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>105 (100%)</td>
</tr>
</tbody>
</table>

Table 2 shows that the lip prints pattern in boys’ subjects, 16.7% were inherited from their father, 38.9% from mother, and 44.4% of the inheritance pattern couldn’t be determined precisely. The Spearman correlation test showed that there was no significant correlation of the lip prints pattern between boys and their parents, \( p > 0.05 \). On the other hand, the inheritance pattern of lip prints was observed in girls’ subjects, with 38.5% were inherited from their father, 46.2% from mother, and 15.4% tends to have a different type from their parents. The Spearman correlation test shows a significant correlation of lip prints pattern between girls and their parents, \( p < 0.05 \).

Table 2: Correlation test of the lip print pattern inheritance

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Girls</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Correlation</td>
<td>Father</td>
<td>Mother</td>
<td>Non</td>
<td>N</td>
<td>Correlation</td>
<td>Father</td>
<td>Mother</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>Spearman</td>
<td>16.7%</td>
<td>38.9%</td>
<td>44.4%</td>
<td>26</td>
<td>Spearman</td>
<td>38.5%</td>
<td>46.2%</td>
<td>15.4%</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>Sig.</td>
<td>0.2441</td>
<td>0.1306</td>
<td></td>
<td>26</td>
<td>Sig.</td>
<td>0.0162*</td>
<td>0.0388*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*indicate the significant correlation
The correlation between the lip prints pattern and the blood group type were also statistically analyzed, as described in Table 3. Most of the subject with type II of the lip print pattern tends to have the blood type A, 14.3%. However, the fisher exact test indicates that the correlation of the lip prints pattern and the blood group type was not statistically significant, \(p>0.05\).

<table>
<thead>
<tr>
<th>Lip Print Type</th>
<th>Blood Group Type</th>
<th>Fisher Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (N = 30)</td>
<td>B (N = 31)</td>
</tr>
<tr>
<td>Type I (N = 22)</td>
<td>3 (2.9%)</td>
<td>7 (6.7%)</td>
</tr>
<tr>
<td>Type I’ (N = 16)</td>
<td>7 (6.7%)</td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td>Type II (N = 36)</td>
<td>15 (14.3%)</td>
<td>9 (8.6%)</td>
</tr>
<tr>
<td>Type III (N = 17)</td>
<td>3 (2.9%)</td>
<td>7 (6.7%)</td>
</tr>
<tr>
<td>Type IV (N = 14)</td>
<td>2 (1.9%)</td>
<td>6 (5.7%)</td>
</tr>
<tr>
<td>Type V (N = 0)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Individual identification is an essential proceeding to assist the process of recognizing the victim and the suspected person’s identity in criminal cases. Many studies have shown that lip prints are unique and remain unchanged during a lifetime. In some cases, such as sexual abuse, the lipstick and bloodstains are often found in a crime scene and become a piece of evidence that correlated with the victim or the suspected person. Untraced lipstick stain on any objects may be easily lifted by aluminum and magnetic powder [11–18].

In the present study, type II was found to be the most predominant lip prints pattern in males and females, and type V has not appeared in all subjects. The result also indicates that the lip print patterns were inherited from parents to the offspring in the Javanese population. The lip prints pattern of girls tends to be inherited from their mother; however, these inheritance patterns couldn’t be determined precisely in boys’ subjects. These results are in agreement with the previous study conducted by George et al in 31 families of the Malaysian population [18,19]. Another study by Arisetiawan (2014) showed that the lip prints pattern of girls tends to be inherited from their father, and boys from their mother [20].

The statistical analysis shows that there was no significant correlation between the lip prints pattern and
blood group type. These results correspond with the previous studies in India with the Ancestral North Indian population sample by Karim and Gupta (2013), which stated that there was no significant correlation between lip print patterns and blood type ⁴.

Many aspects, such as the duplication technique and observer subjectivity, were considered as the influence factor in the study of lip prints pattern. There are several techniques of making a lips impression, such as conventional technique and photographic assistance ²¹. This study used a simple conventional technique which has some limitation related to the problem during the lipstick application, hand pressure, and the variety of lipstick products.

**Conclusion**

There is a resemblance of the lip print patterns between parents and their offspring. The lip prints pattern of girl subjects tends to be inherited from the mother, while for boys, the inheritance pattern couldn’t be determined precisely. There is no significant correlation between the lip prints pattern and blood group type. Further studies with larger samples number are suggested to obtain more accurate results.

**Source of Funding:** Privately funded by the author

**Conflict of interest:** Nil.

**References**


Human Age Estimation Through DNA Methylation Analysis Method from Bite Mark Samples in Forensic Identification

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Abstract

Background: Age estimation are often calculated using bone and teeth samples, but are limited to human skeleton findings. The aim of this research is to estimate human age through DNA methylation method from bite mark samples. Result: There were 40 bite mark samples obtained from healthy volunteers. DNA samples were isolated using DNAzol reagent and converted using DNA methylation kit. The isolated DNA were amplified and electrophoresis was conducted using agarose gel. Electrophoresis result was used as length reference for the sequenced band and analyzed for methylation percentage and correlation with age estimation. Statistical test showed that there was a significant correlation between DNA methylation percentage with age estimation both in men (r Pearson 0.767) and women (r Pearson 0.878). Conclusion: CpGplot emboss analysis for DNA methylation mean percentage in men tend to increase in accordance to age categories, whereas for women, the mean DNA methylation percentage in age categories was stable or constant.

Keywords: Age estimation, DNA methylation, Bite mark

Background

Forensic identification helps investigators determining a person’s identity. Personal identification is often a challenge in both criminal and civil cases. Determining personal identity appropriately is very important in investigations because errors might bring fatal consequences in judicial process.1

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E-mail: yudi4n6sby@yahoo.co.id
Tel +62813-3019-8281

Forensic medicine plays a crucial role in identification, especially for unidentified corpses, damaged corpses, decomposed corpses, burnt corpses and corpses that were involved in mass accidents, natural disasters, riots that resulted in many deaths, as identification for pieces of human corpses or skeletons or others. Forensic identification also plays a role in various other cases such as kidnapping of children, swapped babies, or babies with questionable parents. A person identity can be confirmed if at least two methods give positive results (no doubt).2

In certain cases, for example sexual assault, bite marks are often found. The presence of physical evidence such as bite marks in rape, murder and violence cases is crucial. Bite marks are the most common evidence in rape cases. This evidence also plays a role to determine the physical violence type and the offender’s age.3,4
Suspect identification process using bite marks as evidence is generally carried out by comparing photo interpretation with teeth model of the suspected offender, including analysis and measurement of size, shape and position of each tooth. However, this technique does not always bring accurate results, therefore another way to identify bite marks is conducted through irrigation technique on bite marks to conduct DNA identification. DNA samples from bite marks could be taken from saliva, attached stains, mucosal epithelial remains in saliva, and others.

Age estimation using biomolecular approach can be carried out based on analysis of cell, mitochondrial DNA (mtDNA) sequences’ changes and epigenetic changes, which do not affect DNA. Oral cavity as well as teeth tissues have various cells that could be used as genetic material source. Several methods that might be used to determine chronological age from teeth samples include aspartic acid racemization (AAR), mitochondrial DNA mutations, telomere shortening, advanced glycation end-products (AGEs), and DNA methylation. DNA methylation is a modification process of 5 ’carbon atoms in cytosine residue followed by guanine residue, therefore it is called as CpG(s)/CpGsites dinucleotides. Replication process in DNA methylation is only found at 5 pyrimidine rings position from cytosine in CpG dinucleotide sequence. DNA methylation is the best epigenetic modification method for estimating age from human biological samples. The purpose of this study was to determine age estimation through DNA methylation analysis from bite mark samples.

**Methods**

**Methods and samples**

This study was an analytic cross-sectional design observational study. The samples in this study were bite marks on glasses used by 40 volunteers who have assigned their informed consent, consisting 20 men and 20 women ranging ages from 5 to 65 years. This study has received ethical eligibility from Faculty of Dentistry which number: 350 / HRECC.FODM / VII / 2020.

**Sample preparation**

A total of 30 bite mark samples were stored in conical tubes/dinking glasses (figure.1) and were labeled with 1-40 (sample number’s order)-XY/XX (sex)-category (according to WHO). Human age categories according to WHO (Depker RI, 2009) is divided into four groups: children (5 - 11 years), adolescents (12-25 years), adults (26-45 years) and elderly (46-65 years).

**DNA extraction**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Age (Years Old)</th>
<th>Sex</th>
<th>Concentration DNA bite marks (ng/µl)</th>
<th>Purity DNA bite marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>5.0</td>
<td>L</td>
<td>31.45</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>L</td>
<td>37.56</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>P</td>
<td>45.34</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>L</td>
<td>56.25</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>P</td>
<td>56.15</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>P</td>
<td>39.36</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>7.8</td>
<td>L</td>
<td>78.65</td>
<td>1.35</td>
</tr>
</tbody>
</table>
### Table 1. Measurement of Sample Concentration and Purity DNA

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Youth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.3</td>
<td>L</td>
<td>45.38</td>
<td>1.12</td>
</tr>
<tr>
<td>13.4</td>
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<td>67.56</td>
<td>1.22</td>
</tr>
<tr>
<td>13.7</td>
<td>P</td>
<td>68.74</td>
<td>1.34</td>
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<tr>
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<td>P</td>
<td>78.64</td>
<td>1.54</td>
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<tr>
<td>15.0</td>
<td>P</td>
<td>76.64</td>
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<tr>
<td>16.7</td>
<td>L</td>
<td>56.45</td>
<td>1.23</td>
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<tr>
<td>17.0</td>
<td>P</td>
<td>35.56</td>
<td>1.21</td>
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<td>P</td>
<td>32.65</td>
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</tr>
<tr>
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<td>L</td>
<td>43.47</td>
<td>1.21</td>
</tr>
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<td></td>
</tr>
<tr>
<td>26.5</td>
<td>L</td>
<td>42.35</td>
<td>1.33</td>
</tr>
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<td>L</td>
<td>34.45</td>
<td>1.31</td>
</tr>
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</tr>
<tr>
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<td>P</td>
<td>65.34</td>
<td>1.31</td>
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<td>30.5</td>
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</tr>
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<td>55.25</td>
<td>1.03</td>
</tr>
<tr>
<td>40.1</td>
<td>L</td>
<td>67.54</td>
<td>1.20</td>
</tr>
<tr>
<td>43.0</td>
<td>P</td>
<td>78.42</td>
<td>1.32</td>
</tr>
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<td>44.2</td>
<td>L</td>
<td>98.23</td>
<td>1.33</td>
</tr>
<tr>
<td>Elderly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.0</td>
<td>L</td>
<td>78.43</td>
<td>1.21</td>
</tr>
<tr>
<td>47.4</td>
<td>L</td>
<td>88.56</td>
<td>1.02</td>
</tr>
<tr>
<td>48.0</td>
<td>L</td>
<td>67.75</td>
<td>1.21</td>
</tr>
<tr>
<td>49.0</td>
<td>P</td>
<td>77.43</td>
<td>1.11</td>
</tr>
<tr>
<td>50.0</td>
<td>P</td>
<td>65.17</td>
<td>1.07</td>
</tr>
<tr>
<td>51.0</td>
<td>L</td>
<td>55.31</td>
<td>1.43</td>
</tr>
<tr>
<td>51.3</td>
<td>L</td>
<td>45.65</td>
<td>1.23</td>
</tr>
<tr>
<td>57.0</td>
<td>P</td>
<td>34.32</td>
<td>1.21</td>
</tr>
<tr>
<td>58.1</td>
<td>P</td>
<td>47.14</td>
<td>1.54</td>
</tr>
<tr>
<td>65.0</td>
<td>P</td>
<td>54.54</td>
<td>1.32</td>
</tr>
<tr>
<td>Average</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>55.78</td>
<td>1.27</td>
</tr>
</tbody>
</table>
Isolation / extraction was carried out using DNAzol reagent. Samples in pellet form inside conical tubes were added 1 ml of DNAzol reagent, both were mixed by vortex, then incubated for 5 minutes at room temperature. Afterwards, 10,000 rpm centrifuge was carried out for 10 minutes at 40°C, the viscous supernatant was taken and transferred into a new tube. The new tube is added 0.5 ml of 100% ethanol (absolute), mixed, and incubated at room temperature for 1-3 minutes, and then centrifuged at 4000 rpm speed for 1-2 minutes at 4°C. Supernatant was removed carefully to prevent accidental removal of DNA (pellets). The pellets were washed with 0.8-1 ml of 75% ethanol 2 times and each time the tube was washed back and forth for 3-6 times. The tube was placed in an upright position for 0.5 - 1 minute, then 75% ethanol was removed by pipetting or decanting. The pellets were dried by leaving the tube open for 5–15 seconds. The pellets containing DNA were dissolved with 25-30 ul of distilled water, and then were vortexed adequately, and then stored at -20°C. Furthermore, DNA level and purity measurement were carried out using a UV Spectrophotometer, the results were mean DNA level and purity, as in table 1 and figure 2.

**Bisulfite conversion (MethylEdge™ Bisulfite)**

Fifty microliter of DNA samples in eppendorf tube were added with 130 µl of CT Conversion Reagent. This mixture was then centrifuged at 5000xg speed for 1 minute. PCR tube was placed in PCR machine with the following setting: 980°C for 8 minutes, 640°C for 3.5 hours, and kept in a storage at 40°C for up to 20 hours. Furthermore, 600 µl of M-Binding Buffer was poured into Spin IC column with samples from PCR results. The column was closed and stirred by turning the column several times. Afterwards, it was centrifuged at 10000xg speed for 30 seconds, then the supernatant was removed. The resulting pellets were washed with 100µl M of wash buffer into the column, and then centrifuged at 10,000xg speed for 30 seconds. The supernatant was removed, and the pellets were added with 200µl M of Desulphonation Buffer, and then incubated at 200°C for 20 minutes. Centrifuged was carried out again at 10,000xg speed for 30 seconds. Pellets were then washed twice with 200µl M of Wash Buffer and then centrifuged at 10000xg speed for 30 seconds. Finally, 10µl M of Elution Buffer was added and centrifuge was carried out at 10000xg speed for 30 seconds.

**PCR amplification**

PCR amplification was carried out using T7 promoter tag primer and 10 mer tags and HotStarTag Plus Master mix kit. The setting was as followed: temperature of 950°C for 5 minutes in 30 cycles (denaturation: temperature of 940°C for 1 minute, annealing: temperature of 650 C for 1 minute, extension: temperature of 720C for 1 minute), temperature of 720C for 10 minutes. PCR result using 1% agarose gel electrophoresis showed bands between 150pb-300pb (figure.3).

**Sequencing**

**Table 2. Percentage of DNA methylation in bite marks samples at a length of 300 bp**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Age (Year Old)</th>
<th>Sex</th>
<th>Length of CpG sites</th>
<th>Number of sequens</th>
<th>Methylation Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>5.0</td>
<td>L</td>
<td>123</td>
<td>298</td>
<td>41.28</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>L</td>
<td>109</td>
<td>278</td>
<td>39.21</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>P</td>
<td>210</td>
<td>298</td>
<td>70.47</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>L</td>
<td>121</td>
<td>278</td>
<td>43.52</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>P</td>
<td>89</td>
<td>296</td>
<td>30.07</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>P</td>
<td>191</td>
<td>296</td>
<td>64.53</td>
</tr>
<tr>
<td></td>
<td>7.8</td>
<td>L</td>
<td>176</td>
<td>289</td>
<td>60.89</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>L</td>
<td>134</td>
<td>279</td>
<td>48.03</td>
</tr>
</tbody>
</table>
The sequencing process required 30 µl of PCR sample to identify CpG sites as methylation age marker. The primer used was T7 with 5-CAGTAATACGACTCACTATAGGGAGAAGGCT-3’ order. The results of sequencing with Applied Biosystems 3130 XL Genetic Analyzers were read with bioedit®. In FASTA form, CpG sites percentage was calculated using an online application, Emboss Cpgplot (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/) (Table.2)(figure.4).

<table>
<thead>
<tr>
<th></th>
<th>Youth</th>
<th>Adult</th>
<th>Ederly</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.2</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.8</td>
<td>P</td>
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<tr>
<td>12.3</td>
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<tr>
<td>13.7</td>
<td>P</td>
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</tr>
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<td>15.0</td>
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<td>16.7</td>
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<td>17.0</td>
<td>P</td>
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<td>22.0</td>
<td>L</td>
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<tr>
<td>32.0</td>
<td>P</td>
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<td>37.2</td>
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<td></td>
</tr>
<tr>
<td>40.1</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.0</td>
<td>P</td>
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<td>47.4</td>
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<tr>
<td>48.0</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49.0</td>
<td>P</td>
<td></td>
<td></td>
</tr>
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<td>50.0</td>
<td>P</td>
<td></td>
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</tr>
<tr>
<td>51.0</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51.3</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57.0</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>58.1</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65.0</td>
<td>P</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Percentage of DNA methylation in bite marks samples at a length of 300 bp
Results

Figure 2. Graph of average DNA concentration in bite marks sample

Figure 2 above shows that average DNA concentration sample from bite marks was 55.78 ng/µl, while DNA purity ranged from 1.02 to 1.65. Electrophoretic DNA visualization with 1% agarose gel revealed DNA bands ranging from 150bp to 300bp.

Figure 3. PCR visualization of bisulfite converted DNA bite marks samples

Figure 3 above shows the results of agarose gel electrophoresis from bite marks samples through DNAzol isolation. Afterwards, isolated DNA were conducted bisulfite conversion using DNA methylation kit from MethyIEdge TM Bisulfite promega. Isolated DNA samples were then analyzed in PCR machine using reagents from HotStarTaq Plus Master Mix with T7 promoter tag primers and 10 mer tags. All samples have bands / bands in 300bp core sequence.

All visualized samples were in 300bp core sequence, therefore sequencing was carried out in 300bp core sequence using Applied Biosystems 3130 XL.
Genetic Analyzers that was read with bioedit® and analyzed on CpG sites using Emboss Cpgplot application. DNA methylation percentage from bite marks samples are presented in table below.

<table>
<thead>
<tr>
<th>Age Criteria</th>
<th>Average % of Methylation DNA based on age criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (5-11 years)</td>
<td>60</td>
</tr>
<tr>
<td>Youth (12-25 years)</td>
<td>50</td>
</tr>
<tr>
<td>Adult (26-45 years)</td>
<td>40</td>
</tr>
<tr>
<td>Senior (46-65 years)</td>
<td>30</td>
</tr>
</tbody>
</table>

**Figure 4. Graph of average percentage of DNA methylation based on age criteria**

Figure 4 shows average DNA methylation percentage in men tends to increase in accordance to age categories, whereas for women the DNA methylation percentage is stable or constant. This is most likely to be influenced by lifestyle, environmental, and previous disease factors.

**Discussion**

In genome, methylation occurs in a CpG-rich region named CpG island. Protein and methyl-CpG bonding domains will bind specifically to CpG island, inducing repression and transcriptional activation.

As humans get older, DNA methylation pattern gradually shows DNA hypomethylation and hypermethylation on a specific CpG island-associated promoter. Correlation between DNA methylation and aging can be described in two parts, which are epigenetic drift and epigenetic clock. Epigenetic drift shows a variety of changes in individuals that are associated with age due to environmental factors, on the other hand, epigenetic clock is only markedly related to age, therefore it might be used to predict an individual chronological age.

Between male and female, CpG site has different DNA methylation rates. CpG site is located at X chromosome, therefore differences in sex-specific methylation at X chromosome tend to be more unstable. Women susceptibility to stress and certain diseases also greatly influences DNA methylation rate, thereby factors that influence DNA methylation are widely variable between individuals. Degenerative diseases and metabolic syndrome of each individual also greatly affect DNA methylation percentage. The course of degenerative disease targets cellular epigenetic machine elements, altering epigenetic machines expression and activity therefore inducing epigenetic changes in each individual.

DNA methylation process might turn into a dynamic process, where individual hormonal state greatly affects cytosine hydroxymethylation role. The use of certain oxidative and antioxidant drugs will also affect histone modification rate. This might occur, for example, via cytosine mutations in case of methylation on normal cells. Methylated cytosine absence might lead to permissive histone modification and allow genes to be expressed. Unlike genetic mutations, epimutation does not alter DNA basic sequence and is potentially reversible. Certain diseases such as neoplastic, degenerative, metabolic, and inflammatory might cause oxidative stress that affects gene activation, such as...
epigenetic-DNA methylation.15

**Conclusion**

Mean DNA level from bite marks was 55.78ng/µL. There was a significant correlation between DNA methylation percentage (CpG sites) from bite mark samples with human age estimation both in men and women.

**Sources of Funding:** The author and the co-authors acknowledges to Rector of Universitas Airlangga that has given the funding to the program which called Hibah Penelitian Tesis Magister which number SK no 27/E1/KPT/2020.

**Conflict of Interests:** The author and co-authors declare that they have no conflict of interest in publishing this article.

**References**


Evaluation of Cusp Deflection in Teeth Restored with Various Manipulation Techniques and Types of Composite Restorations

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Abstract

This study aim to evaluate and compare the deflection of premolar teeth cusps that filled incrementally with conventional composite (Universal Ceram X One Sphere TEC TM) and various types of bulk fill composite materials (Kerr SonicFill™3 , 3M Filtek™ Bulk Fill and Shofu Beautifil Bulk Fill). In this study 40 human, healthy maxillary first premolars were collected and large MOD cavity were prepared in them. According to restorative materials, the teeth were haphazardly categorized in 4 groups (n=10 per group). Group I : The teeth were filled with SonicFill™3 Kerr composite, Group II : The teeth were filled with Filtek Bulk Fill, Group III : The teeth filled with Shofu Beautifil Bulk Fill composite, and Group IV : The Teeth were filled with Dentsply Ceram X One Sphere TEC TM. By the aid of digital microscope intercuspal distance on the tips of the cusps between two index reference points was measured pre preparation of the cavity post preparation, after 15 minutes of completion of fillings. The cuspal deflection was calculated by determination of the changes in measurements. Inward cuspal deflection was occurred in all teeth after filling, minimal cuspal deflection reported in all study groups that filled with bulk fill filling in comparison with group IV that filled in layering technique with conventional composite. Greater cuspal deflection produced by Beautifil Bulk Fill restorative in comparison with other groups. As a conclusion of this study that using of new bulk filling restorative materials could dramatically minimize cuspal deflection.

Keywords: Deflection in the cusp, Ceram X One Sphere TEC ™, SonicFill™3

Introduction

The demands for tooth color restore are increased nowadays so Commonly composite filling materials is used for direct restoration for posterior teeth (1). However, the polymerization shrinkage when using direct composite resins is considered a major drawback which is prompted by curing of the composite resin matrix (1).

Two clinical problems (cuspal deflection and microleakage) are associated with the polymerization shrinkage stress. Bonding strength results from the polymerization shrinkage stress is higher than the bonding strength of the adhesive material, which may lead to marginal Gap creation and failure of the composite-tooth interface. Thus Can lead to sensitivity after restoration and development of secondary caries, while when adhesion bonding strength is sufficient to withstand the stress of polymerization; cuspal deflection occurs and can cause tooth fracture, cracks in the enamel, this described by the patient clinically as pain and sensitivity postoperatively (2).

Two major biomechanical factors are influencing the type and the amount of deflection of the cusp, the previous geometrical factors (involve cavity wall thickness post preparation and dimension of the cavity) and the properties of the material (polymerization shrinkage, elastic modulus, the flow of the material...
and hygroscopic expansion), this representing the first factors group; whereas the second factors group is clinical one; including the techniques of restoration placement, indirect or direct technique of restoration, low modulus of elasticity liners as layers to absorb stress, and the system of light curing and the protocol of usage (3,4).

In large cavities, the golden standard technique for resin composite placement is Incremental layering technique since it is capable of minimizing the effects of stress related to shrinkage and enable an acceptable conversion level (5).

The introduction of new kinds of restoration materials (Bulk fill composites restoration) which minimize the needed Placement time. The layer thickness could be 4-5mm of these materials with adequate polymerization and low polymerization shrinkage stress (6). Indeed, the data which is available about these restoration materials influence of on cuspal deflection is little. The aim of this study was to evaluate the deflection of the cusp by restoration of maxillary first premolars with advanced new kinds of bulk fill restoration materials and make a comparison between their results with incremental layering technique by using conventional composite material.

Materials and Methods

In this study forty non carious, healthy human max. first premolar teeth were gathered. The aim of teeth extraction was for orthodontic cause, they stored instantly in special container with distilled water. Any calculus deposits were carefully removed from the teeth with air sonic scaler, washed and polished by using pumice. Cracks examination was done for all selected teeth through visual examination with the aid of magnifying lens and using transillumination from light cure unit. The roots of each tooth have been mounted by the use of dental surveyor vertically into custom made silicon mold. 3M single bond universal used to bond two heads of pins as a reference points into indentation prepared by small round bur on the Buccal and palatal cusps tips of each tooth. After that high speed hand piece with water coolant used to prepare each tooth to large MOD cavity by the use of flat ended fissure bur. To confirm standardization of cavity a modified dental surveyor was used to control the motion of hand piece in all preparation, to ensure high quality of cutting burs were discarded every five preparations. The preparation of each cavity was 3 mm depth and 3 mm width at gingival seats of the boxes and at the pulpal floor. The cavo surface cavity margins were created at 90° with cavity rounded internal line angle.

Three M universal single bond was used in etch and rinse technique in each prepared tooth. Next, according to kind of restoration, the teeth divided into 4 groups; Group I: The teeth were filled with Kerr SonicFill™ composite. Group II: The teeth were filled with 3M Filtek Bulk Fill composite in the same manner of Group I. Group III: Teeth were filled with Beautifil Bulk Fill in one bulk increment same to Groups I and II. Group IV: the teeth were filled in multiple increments of with Universal Ceram X One Sphere TEC™ in 2 mm of each layer thickness. Following the manufacturing instructions, The curing time of each increment was twenty seconds. The measurement the intercuspal distance of each sample was done with the aid of Dino lite digital microscope.

Intercuspal distance was calculated at three different times: for intact tooth, after finishing the preparation of each tooth, and the last record was done in 15 minutes after tooth restoration. Cuspal deflection after the preparation of cavities (CD1) was determined by intercuspal distance subtraction after the cavity preparation from the intercuspal distance for unchanged teeth. Initial distance was recorded from the intercuspal distance post preparation, whereas the final distance was recorded from inter cuspal distance after 15 minutes (7). After that, subtraction of final distance from initial distance was done to measure the cuspal deflection that happened as a consequence of polymerization shrinkage stress (CD2).

Results

Inward cuspal deflection (CD1) and (CD2) measured in (mm) micrometer units (descriptive statistics) are shown in the table no. 1.
Table 1. Inward cuspal deflection (CD1) and (CD2) measured in mm (descriptive statistics)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD1</td>
<td>CD2</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>4.5982 ±1.2105</td>
<td>5.8315 ±0.5332</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>4.4121 ±1.3134</td>
<td>5.7827 ±1.2185</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>3.9671 ±0.9543</td>
<td>7.2637 ±1.2641</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>3.4285 ±1.1842</td>
<td>9.5743 ±1.5432</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.469</td>
<td>0.000</td>
</tr>
</tbody>
</table>

All groups post cavity preparation showed no significant differences in CD1 using one way ANOVA with P value > 0.01). Although, after 15 minutes of complete the filling, Significant difference was reported among all study groups (P = 0.000).

**Discussion**

The common problem of composite restoration is cuspal deflection due to the resin-based composite stress on the surface of the tooth, which could serve as a preload encouraging tensile fractions of the tooth and could be the key cause of failure during the curing of the composite (8,9).

The deflection of the cusp was assessed by using of extracted teeth since the problem of supporting structures and the testing system compliance have been eliminated (10). In this study, Maxillary first premolar teeth were used because they are evenly sized and shaped (11).

Then, large MOD cavity was prepared to get high C factor ,the remaining tooth structure weaken, and cause 63% of the cusp strength loss ; As a result, possibility of the deflection of cusp is increased (12,13) . When comparing MO or DO against MOD fillings ,the shrinkage strength of the composite polymerization can cause lower negative deflection in cusps because minimum amounts of restorative material are required (14–17).

The cuspal deflection was estimated after 15 minutes of finishing restoration by measuring the distance between both reference points which was longer and slower than other composites in the polymerization shrinkage, through this time the largest degree of inward displacement was happened as reported by many previous studies (18–20).

This is due to the remaining free radicals. There have continued to react double bonds in composite restoration. Therefore, after complete polymerization, For several minutes the deformation continued (21,22).

These finding was consistent with other studies that conclude that after polymerization, an inner cuspal deflection occurred because of polymerization shrinking stress development (7,23–26).

The results of this study is defection in the cusps deflection when compared with conventional type (6,25–30). The addition of stress relievers to the bulk fill composite alter the dynamics of shrinking, decreasing resin matrix with Increased loading of the filler, and thus minimize polymerization shrinkage stress and decrease in cuspal deflection (26,28,30).

On the other hand, this disagree with other studies, which has shown that there is no difference between bulk resin composites and conventional composites in shrinkage stress composites and cuspal deflection (31).

After restoration, minimal mean value of cuspal deflection had been reported in teeth filled with Filtek™ Bulk Fill (group II), removal of TEGDMA monomer from its resin compartment might be the cause for that.
In this study, there is no statistical significant difference between teeth filled with SonicFill™3 (group I) and teeth filled with Filtek™ Bulk Fill (group II). Filtek™ Bulk Fill and SonicFill™3 composites in order to minimize shrinkage stress because their manufacturers have revolutionized their manufacturing mechanism and their monomers composition respectively. In this study, greater cuspal deflection reported in teeth filled by Beautifil Bulk Fill (group III) than other groups of bulk fill kinds used, because of the presence of high molecular weight polymerization modulators which minimize polymerization shrinkage in the remaining groups of bulk fill composite material that had been used in this study.

Conclusion

Bulk fill resins-based material resulted in lesser cuspal deflection in comparison with conventional incremental composite. In Bulk fill composite material the cuspal deflection magnitude is related and affected by the kind of composite restoration materials.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References


The Role of Health Education in Promotion of Health Care Waste Management in Khartoum North Teaching Hospital

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Abstract

The study was conducted as a quasi-experimental design in Khartoum North teaching hospital to assess the role of health education in the promotion of health care waste management. A sample size of (103) targeted workers (cleaners) were used in the investigation. Data collected was analyzed using the T-test and chi-square test by computer using SPSS. The results revealed remarkable improvement in the knowledge of the respondents, as regards the hazards of the health care waste (53%-100%) before the intervention, after the intervention, it increased to 100% showing highly significant change. The opinion of the respondents to use the protective clothes ranging from (45% - 100%) before the intervention, rose after the intervention to (70.5% – 100%) showing significant change. The practice of workers about touching contaminated cotton and linen with patient body fluids was 16.7% before the intervention, after the intervention only 7.4% touch the waste showing significant change.

Keywords—Health Education, Health Care Waste Management, Health workers, Health Promotion.

Introduction

Health care waste includes all the waste generated by health care facilities, research facilities and laboratories. It includes the waste originating from minor or scattered sources such as that produced in the course of health care under taken in the home (dialysis, insulin injection etc.) (¹). Hazardous health care waste is classified to: Sharps, non-sharps blood, body parts, chemicals, pharmaceuticals, medicals, devices and radioactive materials (²). WHO stated that, between 75% to 90% of health care waste produced by health-care providers is non-risk or “general” health-care waste, it is comparable for domestic wastes. The remaining 10-25% of health care waste is regarded as hazardous, may be infectious, toxic, or radioactive (³). Health care waste is a reservoir of potential harmful major organisms which can infect hospital patients, visitors, health-care workers and the general public particularly the rage pickers (⁴).

Integrated waste management systems for health care waste are not introduced in most developing countries resulting in mixing of hazardous and nonhazardous waste (¹). Poor management of health care waste causes serious diseases in health care personnel, waste workers, patients and the general public. The main sources of illness from infectious waste is probably due to needle stick injuries, which can cause hepatitis B and C and an estimated 250000 cases of HIV. There are, however numerous other diseases that could be transmitted by contact with infectious health care waste (⁵). WHO stated that the main reasons for failure of waste management are absence of effective waste management, lack of awareness about the health hazards, insufficient financial and human resources, poor control of waste disposal, and many countries do not have appropriate regulation or do
not enforce them (4). These are to some extent the same reasons for the failure of health care waste management in Sudan which it is still treated as municipal waste concerning collection, storage, transportation, treatment and disposal (6).

In many health facilities the health care waste is handled in the same manner as domestic refuse and collected in black bags and this is unsafe and mishandling, collection, storage movement transportation and disposal practices e.g. the waste collected in open container and full until the waste scattered in the ground. Central Storage areas in many health care facilities may provide breeding sites for vectors for many diseases and provision of the food for cats (7). Comprehensive risk assessment of all activities involved in health care waste management carried out aiming to promote health care waste management, will allow the identification of necessary protection measures, these measures should be designed to prevent exposure within safe limit. Once the assessment is completed health staff should receive suitable training and increasing the awareness is indispensable in this program which could be achieved via information dissemination thus health promotion expected to play leading role in the protection of health staff.

The preset study aimed that to raise the awareness of workers to words health care waste management and potential risks associated with health care waste. To assess the knowledge, attitude and practice of the cleaners about health care waste management. To encourage the usage of protective measures to protect the workers from the hazards of health care waste.

Materials and Methods

Study Area: Khartoum North Teaching hospital was founded 1965 in area of 65000 (Km)^2, and consists of 15 department.

Study design: Quos experimental [interventional. hospital based study

Study population: Cleaners (325) in Khartoum North Teaching Hospital

Sample size: The sample size was determined from Khartoum north teaching hospital cleaners by the formula: N= z^2 p q d^2 / d^2 Sample size = (103 worker)

Sample selection: The individual of the study population in the hospital were selected using the systematic random sample from the total.

Phases of the stud:

Phase One: (Data collection)

Preliminary survey was conducted in order to assess the knowledge, attitude and practice of the cleaners using these methods.

Questionnaire: The questionnaire contains the following variables wastes segregation, packaging, safe handling, storage, color coding and collection of the medical waste. A questionnaire was prepared to assess the knowledge, attitudes and practice of the cleaners towards the health care waste. needle stick –injury, hepatitis infection, immunization, protective cloth, regular examination and the knowledge of the hazards of health care waste.

Interviews: Hospital managers interviewed for their responsibilities toward hospital safety and what is their policy program, planning to improve the hospital hygiene and the workers their training, protective measures, laws, regulations and rules governing the staff.

Reports: Reports are collected on information of health care waste management program in the hospital about reported cases of infectious diseases, regular medical examination, immunization and needle stick injury among the workers.

Observation: personal observation focused on health care waste management and the workers practice before and after the intervention.

Phase two: (intervention)

Training courses: Training courses was held for workers, two days the training courses included:

Lecture 1- Health care waste management. Lecture 2 –Health care waste hazards.

Lecture 3 –Health protection and healthy practice for workers

Audiovisual aids: Audiovisual aids e.g. Video tape and posters used during lectures of training courses
and workshop for workers to reinforcing the lectures and papers, video tap+ was used on from World Health Organization from Al Bashir hospital in Jordan.

**Phase three: (Evaluation)**

Evaluate knowledge, attitude and practice of the health workers through questionnaire and observation, to compare between pre and post questionnaire to assess effect of health education.

Revising records of intervention phases to evaluate health education activities.

**Data Analysis**

The study used frequency distribution, percentages, figures and tables, chi square test and T test to assess the effective of the intervention phase and comparison between pre and posttest and tested statistically to show the significant change, to test the correlation between two samples. The data was analyzed by computer using SPSS.

**Results**

One-day workshop which was held in the Khartoum North Teaching hospital came out with recommendations. these recommendations have to be implemented in the hospital. The first step is to designate a responsible person (public health inspector) and committee. The committee first focused on the safe practices and procedures for health care waste segregation, segregation of health care waste into three categories general waste in the black pages and hazardous waste in the yellow labeled pages and sharps in the safety boxes. Second step internal collection and storage, the committee provided a small car for collection and transportation of the health care waste from the wards to the storage area, new storage area was built.

**Interviews**

Hospital managers interviewed before the intervention, in the hospital there is no system of the health care waste management, the staff were not trained and did not use the protective cloth and no laws about medical waste management. After the intervention there were clear system of management. The law of Sudan 2009 was provided

- **Reports**

Before the intervention there were no careful records of information of health care waste management concerning, cases of infections, medical examination, immunization and needle stick injury among the workers after the intervention all of them were fully implemented due to the impact of health education.

- **Observations**

Before the intervention no segregation. After the intervention the waste were segregated into three categories general waste in black pages, hazardous waste in red pages and sharps in the safety boxes which were collected from the wards with small car to the storage area. Storage area before the intervention an open area there were uncapping syringes scat red, after the intervention the storage area was constructed away from food storage, preparation of the food, it was easy to clean with smooth finishing, good light and ventilation.

![Figure 1 Distribution of workers in the units of the hospital (N = 103).](image)
Figure 1 shows the distribution of workers in the units of the hospital. More than third of workers are illiterates (38.9%) and more than half of them are have primary education (57.4%). More than half of the workers are in the wards (55.6%). More than one third (35.2%) of the were provided with special bags before intervention. After intervention all sites (96.3%) were provided with special bags. T test = 7.968, p-value = 0.00 showing highly significant change. Before the intervention (16.7%) of the worker used to touch the contaminated cotton and linen, soiled by the patient’s fluids. After the intervention workers minimized the touching of body fluids of the patient. T test = 2.837 p. value = 0.006 showing highly significant change.

Less than half (48.1%) of the workers were exposed to needle stick injury before the intervention and after the intervention there is no new cases of injury. About (13%) of the workers were examined before intervention. After the intervention about the half (48.1%) of the worker were examined due to the impact health education test = 2.214 p value = 0.031 showing significant change. Only (11.1%) of the workers were immunized before the intervention.

Table (1) Shows the co-relation factor between education level and the knowledge of the workers about hazard of health care wastes. (N = 103), $\chi^2 = 26.491$, p- value = 0.000

<table>
<thead>
<tr>
<th>Level education</th>
<th>Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No %</td>
</tr>
<tr>
<td>Illiterate</td>
<td>20 37.0</td>
</tr>
<tr>
<td>Primary</td>
<td>31 57.4</td>
</tr>
<tr>
<td>Secondary</td>
<td>2 3.77</td>
</tr>
<tr>
<td>Total</td>
<td>53 98.1</td>
</tr>
</tbody>
</table>

After the intervention, the half (48.1%) of workers were immunized due to the impact of health education. T test = 4.595 p. value = 0.000 showing highly significant change. Less than half (46.3%) of the workers used protective clothing. After intervention more than three quarters used protective clothing. T test = 2.440 p. value = 0.018 significant change. One fifth (22.2%) of the workers were trained before the intervention. After the intervention all the workers showed a positive respect and were involved in the training program. T test = 2.982 p. value = 0.004 showing highly significant change. More than half (53.7%) of the workers know the hazards. After intervention all (98.1%) of the workers have gained increased knowledge about hazards of health care wastes. T test = 6.512 p. value = 0.000 showing highly significant change.

Table (2) Shows the co-relation factor between education level and the practice of the workers in the means of the collection and transportation of health care waste. (N = 103), $\chi^2 = 27.462$ p- value = 0.000

<table>
<thead>
<tr>
<th>Level education</th>
<th>Practice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheeled trolleys</td>
</tr>
<tr>
<td></td>
<td>No %</td>
</tr>
<tr>
<td>Illiterate</td>
<td>3 5.6</td>
</tr>
<tr>
<td>Primary</td>
<td>2 3.7</td>
</tr>
<tr>
<td>Secondary</td>
<td>0 0</td>
</tr>
<tr>
<td>Total</td>
<td>5 9.3</td>
</tr>
</tbody>
</table>
This means there is co-relation between the level of education of the workers and the knowledge of the workers about hazards showing highly significant test. This means there is co-relation between the level of education of the workers and the practice of the workers in the way of the collection and transportation of health care waste showing significant test.

Table (3) shows the co-relation factor between education level of the workers and the utilization of protective clothing. (N = 103), $X^2 = 1.793, p$- value = 0.408

<table>
<thead>
<tr>
<th>Level education</th>
<th>Utilization</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>illiterate</td>
<td>10</td>
<td>18.5</td>
<td>11</td>
<td>20.4</td>
<td>21</td>
</tr>
<tr>
<td>Primary</td>
<td>15</td>
<td>27.8</td>
<td>16</td>
<td>29.6</td>
<td>31</td>
</tr>
<tr>
<td>Secondary</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3.7</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>46.3</td>
<td>29</td>
<td>53.7</td>
<td>54</td>
</tr>
</tbody>
</table>

This means there is co-relation between the level of education of the workers and the utilization of protective clothing showing in significant test. Because there are other factors e.g. enabling factor, availability and uncomfortable to wear in a hot climate

**Discussion**

Educational attainment of the workers, more than third of workers are illiterate 39.9% and more than half 52.4% of them are primary education which increased the difficulties of the training and explaining of the softly practices proper handling and use of protective cloth. Nazir Mirza 1996 stated that the health workers in Nerobi were over worked, poorly educated and lacking in supervision (8). and also, Shereen Eassa 2007 mention that in Zagazig Egypt the highest risk was observed among unskilled workers back (9). Mona Gamal Eldin (2000) in Egypt illustrated that the level of awareness among hospital workers especially those involved in waste handling is poor. Due to the high illiteracy rate of hospital workers handling waste and lack of supervision, changing their attitudes requires intensive planning and reorganization (8,10). Distribution of the workers in the wards, about three quarters 74.1% were employed in the hospital wards. The type of the work of the workers all of them collect waste in the bags Mamdouh H. M. Abdon (2007) mentioned that in Jeddah (Saudi Arabia) Majority of the workers are poor ignorant and work in any job regardless the hazards that may result from such work (11).

About the third 35.2% of the sites are provided with specialized bags before the intervention. After the intervention all of the sites are provided with specialized bags, showing significant change. Nasir Mirza 1996 in Narobi illustrated that disposal of the waste in an expensive exercise. The plastic bag required are costly and sometimes, simply unavailable (8). Also, Gihan et al. in 2005 in Alexandria stated that segregation in limited amounts of colored bags the remaining are mixed with domestic waste poor segregation due to lack of staff awareness (12). Also, Isam and Rana. Elkhatib (2006) in Palestinian illustrated that management practices were in adequate, there was in sufficient separation between hazardous and nonhazardous wastes (13).

Practice of touching contaminated cotton and linen with blood and other liquid of the patient and the placenta showing highly significant change which agree with, Khalid Youzif and Youzif Alameen (2018) in Sudan mention that the study population handle health care waste both in Khartoum and Bahri hospital 96%use gloves (14). Also, Ahmed and Shuwaiter (1995) in United Arab Emirates stated that biomedical waste in the hospital are primarily segregated from nonhazardous waste they are collected either in yellow bags or syringes boxes (15).
The most common form of occupational exposure experienced by health care staff and waste handlers is by pathogens in blood such as hepatitis B and C, and HIV through a needle stick injury. In Khartoum North teaching hospital, about half of workers had been picked with needle injury (48%) and there were some cases of hepatitis (13%) were reported. Musa (2014) in the Sudan stated that (58.3) of the study population have been exposed to the needle stick injury during the work in Omdurman hospital (16). Sadoh et al. mentioned that sharps injuries have become one of the most important occupational injuries and routes of contagion among health care workers (17). Also, Henry and Campbell (1995) mention that over 40% of the workers had been picked with needle while handling health care waste (18). Also, Laymer U.B (1997) in Sweden stated that needle stick injuries constitute the leading cause of job-related injuries in hospitals (19).

Abd El-Salam (2010) mention that health care workers collectors in Cairo university hospitals, it was found that HBV and HCV infected about 55% at the end of the monitoring period almost a year, also it was found that the prevalence of infection was directly related to the efficacy of the preventive measures specially vaccination and post exposure prophylaxis (20).

Regular medical examination for the workers before or during employment only 13% of the workers were examined before the intervention. After the intervention, the half of the workers were examined due to impact of health education. Henry and Campbell (1995) mention that no medical examination for workers before or during employment (18). Medical surveillance should be implemented for at risk hospital staff, particularly hospital waste collectors, special attention to be paid for hepatitis B, C viral infections. Only about 11% of the workers immunized before the intervention, after the intervention half (48%) of the workers were immunized due to the impact of health education. Ezzadin A.A. Franka (2006) in Libya stated that more than three forth (79%) of health care waste workers are not immunized against hepatitis B (21). Hassan et al. (2018) mention that majority (91.6%) of study population do not receive any vaccination services against hepatitis B (14).

Less than half 46.3% of the workers used protective clothing before the intervention, after the intervention more than three quarters used protective clothing. N Motamed and others (2006) in Iran mention that occupational safety and health regulations require both employers and employees to reduce or eliminate occupational risks protective barrier use is a major element of universal precautions. To encourage their use protective barriers must be readily available, easy to use, effective and comfortable (22,23).

After the intervention all the workers were involved in the training program. Musa (2014) mention that 71.7% of the study population do not received any training about health care waste in Omdurman teaching hospital (16). Hassan et al. (2018) stated that more than half (61.7%) of study population do not received any training in handling infectious wastes (14). And also, mention that only 20% of the study population were trained in Khartoum teaching hospital (6,16) (14).

More than half (53.7) of the workers know the hazards before the intervention, after the intervention all 98% of the workers have gained increased knowledge about hazards of health care wastes showing highly significant change. Graham Ngumi (2006) mention that population of Nairobi Kenya have failed to establish proper facilities for waste disposal and are often unaware of the danger of exposing the community at large to bio hazardous waste (24). Also, Ezzadin A.A. Franka (2006) in Libya stated that 98.7% of the worker having information about AIDS and viral hepatitis, as health risks of the health care waste (21). Ali and others recommended that mass awareness campaign needs to be planned designed and implemented to make the people aware of the consequences of infectious waste handling, storage and disposal. Appropriate communication modes need to be used for this awareness campaign (25).

**Conclusion**

The intervention undertaken achieved remarkable improvement in the knowledge, attitude and practice of the health staff as regards of health care waste management. The Study has suggested that the hazards of health care wastes which have greatly negative impact on occupational and public health. Could be reduced by improved management of hazardous health care waste, safety measures immunization, wearing protective cloth, periodic examination, hand hygiene and awareness raising through training of the health staff and health education for patient, co patient, visitors and
general public. And lastly coordination between medical departments and those involved in the management of health care waste and infection control, all of those are essential component in sustaining the operation of health care waste management system.

### Recommendations

1) To establish clear applicable policy in hospitals to supervise the health care waste management.

2) Ensuring early and proper segregation and identification of hazardous waste through proper color coding and labeling.

3) Establishment a comprehensive occupational health (safety measures, Medical surveillance, Training policy, and safety practice).

### Declaration of Competing Interest

The authors declare that they have no conflicts of interest to disclose. And there is no financial support.

### Funding

No funding for the research.

### Ethical Clearance

Taken from State Ministry of Health.

### References


Effect of Serotonin Concentration and Some Variables in the Sera 6 hours before and after Dialysis on Chronic Kidney Patients with Different Ages in Iraqi Population

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Abstract

This study is concerned with chronic kidney disease and control group . The determine patients from (alseder hospital ) in Najaf city during the period between January 2019 to July 2019. The CKD patient and control group consisted of sixty with age mean of (±20) years. The blood samples were drawn from all patients 6 hours before and after dialysis to measurement serotonin , blood Urea, Creatinine , uric acid and hemoglobin and control group. Also the patients classified into two groups according to age . This variables measured by using assay (ELISA) technique and colorimetric methods. The higher mean value of serotonin was shown in CKD patients before dialysis ( 0.0257± 0.00224 ng /ml) compared with patients after dialysis ( 0.1894 ± 0.05189 ng/ml) compared with control group( 0.0059 ± 0.00139 ng/ml). Also the higher value of blood Urea and Creatinine reversibly in CKD patients before dialysis was (126.25± 4.869 mg/dL), (10.041± 0.3019 mg/dL) compared in blood Urea and Creatinine in CKD patients after dialysis was (48.183± 2.262 mg/dL), (3.28± 0.201 mg/dL) and control group was (28.105 ± 1.896 mg/dL), (1. 095 ± 0.0961 mg/dL).

Also the higher value of uric acid and hemoglobin reversibly in CKD patients before dialysis was (8.0623± 0.1478 mg/dL), (13.800 ± 0.16519 g/dL) compared in uric acid and hemoglobin in CKD patients after dialysis was (5.5361± 0.16055 mg/dL), (12.383 ± 0.15872 g/dL) and control group was (2.6789 ± 0.2977 mg/dL), (13.742 ± 0.18954 g/dL) , all above results there was a significant difference and correlation (p˂0.05).

Aim of study: Study the effect of serotonin, blood Urea, Creatinine , uric acid and hemoglobin 6 hours before and after dialysis on CKD patients and compared with control group .Study the effect of variables on CKD patients with different age and study the relation between CKD patients after and before dialysis.

Key words: serotonin , Before and after dialysis, hemoglobin, uric acid, creatinine

Introduction

The CKD in dialysis the has increased in the diabetes mellitus, chronic obstructive pulmonary disease [1]. Dialysis is considered a good treatment for patients . Serotonin is main neurotransmitters acting on synapses of nerve cells and found in the gastrointestinal tract . The changes serotonin are frequently observed in CKD patients [8]. Uric acid is the a final oxidation metabolite of the purine in the humans, the uric acid level is a risk factor for incident KD in the general human , that serum uric acid levels >7 mg/dl had an increased incidence of KD [8]. The kidney damage is marked by the rise in two chemical substances creatinine and urea is directly toxic and measure of the kidney function [6] . Creatinine is produced from muscles and excreted through the kidneys, creatinine concentration of the serum is maintained by the balance between its generation and excretion by the kidneys [7].
Materials and Methods

The subjects determine patients from (Al-Sadr hospital) in the city of Najaf during the period between January 2019 to July 2019. The CKD patient and control group consisted of sixty with age mean of (80 ±20) years. Also the patients classified into Patient’s age less than 40 years (n=32) and patient age more than 40 years (n=28).

- Procedure of serotonin

In the Serum the concentrations of serotonin in the Serum were determined by using ELISA assays. The ELISA kits for testing serotonin were obtained from Elabscience Biotechnology co. Ltd. The serotonin of number is Product ID is (E-EL-H2187).

- Procedure of blood Urea

In the Serum the concentrations of Urea in the Serum were determined by using colorimetric methods were obtained from abcam Biotechnology co. The Urea of number is Product ID is (ab83362) (at absorbance = 570 nm).

- Procedure of Creatinine

In the Serum the concentrations of Creatinine in the Serum were determined by using colorimetric methods were obtained from abcam Biotechnology co. The Creatinine of number is Product ID is (ab204537) (at absorbance = 490 nm).

- Procedure of uric acid

In the Serum the concentrations of Uric Acid in the Serum were determined by using colorimetric methods were obtained from abcam Biotechnology co. The uric Acid of number is Product ID is (ab65344) (at absorbance = 570 nm).

- Procedure of hemoglobin

In the Serum the concentrations of hemoglobin in the Serum were determined by using colorimetric methods were obtained from abcam Biovision co. The hemoglobin of number is Product ID is (K219-200) (at absorbance = 575 nm).

Statistical Analysis

The statistical analysis was measuring by using SPSS version 17 in which mean, stander error, t-test and ANOVA test were used for data comparison. Correlation between all variables were significant and a p value of <0.05.

Results

The purpose of this study was to evaluate the level of some variables (serotonin, blood Urea, Creatinine, uric acid and hemoglobin) associated with CKD patients 6 hours before and after dialysis and control group are shown in table (1). The higher mean value of serotonin was shown in CKD patients before dialysis (0.0257±0.00224 ng/ml) compared with patients after dialysis (0.1894 ± 0.05189 ng/ml) compared with control group(0.0059 ± 0.00139 ng/ml). Also the higher value of blood Urea and Creatinine reversibly in CKD patients before dialysis was (126.25± 4.869 mg/dL), (10.041± 0.3019 mg/dL) compared in blood Urea and Creatinine in CKD patients after dialysis was (48.183± 2.262 mg/dL), (3.28± 0.201 mg/dL) and control group was (28.105 ± 1.896 mg/dL), (1. 095 ± 0.0961 mg/dL). Also the higher value of uric acid and hemoglobin reversibly in CKD patients before dialysis was (8.0623± 0.1478 mg/dL), (13.800 ± 0.16519 g/dL) compared in uric acid and hemoglobin in CKD patients after dialysis was (5.5361± 0.16055 mg/dL), (12.383 ± 0.15872 g/dL) and control group was (2.6789 ± 0.2977 mg/dL), (13.742 ± 0.18954 g/dL), all above results there was a significant difference and correlation between the mean value in CKD patients before and after dialysis and control group (p<0.05). (Table1).

This result showed the correlation value between some variables (serotonin, blood Urea, Creatinine, uric acid and hemoglobin) in serum of 60 CKD patients before and after dialysis, the result revealed that correlation between serotonin before dialysis and serotonin after dialysis (r = 0.058 (p<0.01) . Also the high correlation between blood Urea before dialysis and blood Urea after dialysis (r = 0.694 (p<0.05) . The result showed correlation between Creatinine before dialysis and Creatinine after dialysis (r = 0.050 (p<0.05) . The result showed high correlation between uric acid before dialysis and uric acid after dialysis (r = 0.241 (p<0.05) . Also the high correlation between hemoglobin before dialysis and hemoglobin after dialysis (r = 0.276 (p<0.05) . (Table 2).
The concentrations of these variables were divided according to different age in CKD patients (Table 3). The patients were divided into two groups according to the age intervals of patients: age less than 40 years, n=32, and age more than 40 years, n=28. The all levels of these variables showed a significant difference in each age group (p < 0.001) (Table 3). This result showed the correlation between serotonin less than 40 years and serotonin more than 40 years (r = 0.268, p < 0.01). Also, the high correlation between blood Urea less than 40 years and blood Urea more than 40 years (r = 0.023, p < 0.05). The result showed correlation between Creatinine less than 40 years and Creatinine more than 40 years (r = 0.488, p < 0.05). The result showed high correlation between uric acid less than 40 years and uric acid more than 40 years (r = 0.135, p < 0.05). Also, the high correlation between hemoglobin less than 40 years and hemoglobin more than 40 years (r = -0.229, p < 0.05) (Table 4).

Discussion

In this report, we showed that the concentration of serotonin was higher in CKD patients before dialysis than CKD patients after dialysis and control groups. The concentration of serotonin in CKD was higher in less than 40 years than other different age. Until now, there are few studies about relation between serotonin and CKD. The increasing serotonin levels and serotonin receptors in patients of CKD [8]. Some factor effect on serotonin level such as depression and age is highly prevalent and is associated with poor quality of life and increased mortality among adults with CKD. Depression is well known to affect adults with end-stage KD [9]. Serotonin has been studied before and after dialysis because we believe there is a relationship between depression and kidney patients and serotonin is a trigger in depression and the risk of hospitalization due to psychiatric disturbances are higher in patients on dialysis in comparison with pre-dialysis and post-transplant patients, the neuropsychiatric manifestations in CKD patients [10]. The Serotonin mechanisms that links with CKD, our resulting shown agree with reports [11]. The creatinine and serum urea was significantly high before dialysis and reduced significantly after dialysis, both serum creatinine and serum urea are widely accepted biomarkers to assess the renal functions [12]. Generally, urea accumulation in blood serum of kidney failure patients arises from the degradation of food and tissues such as muscle [13]. The high level of urea in blood leads the body very sick unless remove it from the blood streams by kidneys. mass differences [14]. Reduction in the level of creatinine during dialysis is also used as a surrogate marker of the inadequacy of dialysis [15]. During the study it was also observed that CKD is more 40 years are more affected with CKD our result agree with [16]. Uric acid level before and after dialysis is measurement and comb with control group our result agree with [17]. It is catabolism and production of uric acid is not limited to dialysis duration and uric acid metabolism measured immediately after dialysis is indicator of uric acid accumulation in the plasma [18].

Conclusions

In CKD patients there were increasing in serotonin, blood Urea, Creatinine, uric acid and hemoglobin in before dialyses compare in after dialyses and control group. Whereas, the serotonin and hemoglobin level were increased in these patients of less than 20 years. While blood Urea, Creatinine and uric acid level showed the highest value in the group of older than 40 years.

Acknowledgment: The Authors present their thanks to all the individuals and institutions who contributed to this work, especially the Staff of the Kidney Center at Al-Sadr Hospital for their assistance in completing this research. Finally, we wish all patients a speedy recovery, God willing.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Taken from Altoosi University College committee

References


Estimation of the Biological Activity of Some Commercial Bleaching Solutions (Hypochlorites) on Pathogenic Bacteria

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Abstract

Hypochlorites, the active ingredients in bleach, are an extremely useful chemical that have been used since the 18th century as a disinfectant. Hypochlorite solutions are in general strong oxidizing agents. The study included of five species of hypochlorite solutions widely used as commercial bleaching solutions (Shoof, Oroplus, Alwazir, Lamoa and Fas) and their concentrations were confirmed chemically by potentiometric titration methods previously and applicate their ability and efficiency against two types of bacteria (E.coli and Staphylococcus arueus) which are isolated from human infected sample in general Heat hospital /Iraq. The results revealed that all bleaching solutions have anti-bacterial influence against two species of bacteria and more successful against Staphylococcus aureus than the E.coli especially with Alwazir solution give high significant difference compared with other solutions, also the outcomes showed sensitivity of bacteria enhanced with concentrations of bleaching solutions. The study indicates the fast reduction rate in the 30S reaction showed the immediate efficiency of Hypochlorites. Finally the current study suggest and encourage to use hypochlorite solutions as antiseptics and disinfectants to killing species of bacteria which proved their efficiency during this study.

Keywords: Bleaching solutions, E.coli and Staphylococcus arueus, Antimicrobial, Microorganism, Hypochlorite’s.

Introduction

Hypochlorites are in general strong oxidizing agents. Commercially, they are used as an alternative to chlorine gas for chlorination of domestic water supplies and swimming pools ¹. They were used also in cooling towers of air conditioners and power stations to control bio fouling ². Acidified sodium chlorite was used an alternative to chlorine in reducing microbial populations to maintain food quality and safety on fresh –cut produce ³. Commercial acidified sodium chlorite and sodium hypochlorite exhibited strong efficiency on reduction of microorganisms including E.Coli ⁴.(Estrela et al) have studied the mechanism of action of sodium hypochlorite on the activity of some types of bacteria. They found that the chloramination reaction between chlorine and amino group (NH) forms chloramines that interfere in cell metabolism ⁵.

Sodium hypochlorite, the active ingredient in bleach, is an extremely useful chemical that has been used since the 18th century as a disinfectant ⁶. In the circulation, NaOCl combines with water to generate hypochlorous acid (HOCI) conferring potent anti-bacterial and antifungal properties ⁷. Some factors affecting the concentration of available chlorine in commercial sources of sodium hypochlorite were studied by (Frias et al) ⁸. The effect of storage temperature and heating on the concentration of available chlorine and pH of 2.5 % NaOCl were investigated and found that storing NaOCl at 4ºC is optimal for longer shelf life and pH of the samples did not affect the stability of NaOCl in this study ⁹. Preheating NaOCl solutions appears to improve
their necrotic pulp tissue dissolution capacity and efficacy against stationary phase of E. faecalis cells. Studies were conducted to compare the effect of sodium hypochlorite (SH) versus mono chloramine (MON) on bacterial populations, these studies indicated that (MON) is superior to SH in reducing microbial populations in poultry chiller water. Antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of E. faecalis were studied, the study confirmed the antimicrobial activity of chlorhexdine and sodium hypochlorite. Other study concluded that heating the solutions have enhanced their ability to dissolve organic material.

**Materials and Methods**

**Preparation of commercial bleaching solutions**

Commercial bleaching solutions are mostly sold in the commercial market in Iraq from different companies such as: Shoof, Oroplus, Alwazir, Lamoa and Fas showed in table 1. Their concentrations were confirmed chemically by potentiometric titration methods before application them against bacteria. The percentages of each solution was converted to milligram units per milliliter. The solutions were then ready to conduct the biological activity of two pathogenic bacteria, namely: *E.Coli* and *Staph aureus*.

<table>
<thead>
<tr>
<th>Name of company</th>
<th>Source</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoof</td>
<td>Iraq</td>
<td>40 mg/ml</td>
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<tr>
<td>Oroplus</td>
<td>Turkey</td>
<td>50 mg/ml</td>
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<tr>
<td>Alwazir</td>
<td>Jordon</td>
<td>30 mg/ml</td>
</tr>
<tr>
<td>Lamoa</td>
<td>Iraq</td>
<td>25 mg/ml</td>
</tr>
<tr>
<td>Fas</td>
<td>Iraq</td>
<td>50 mg/ml</td>
</tr>
</tbody>
</table>

**Culture media:**

They were prepared in accordance with the guidance of the manufacturers and sterilized under pressure of 12-15 PSI in autoclaves for 24 hours at 37 ° C. They were then sterilized. Then used for bacteria analysis culture and diagnosis.

**Culture preparation:**

Bacteria were regenerated and kept in the incubator for 24 hours at an average temperature of 37 ° C. Then they were applied to sterilized tubes with heart infusion broth and put into the incubator at 37 ° C for 24 to 72 hours. The chances of a mild transmission were 27 percent at a wavelength of 580 nanometer and a 100 percent light transmission for the nutrient bread that was used to arrange the micro-organism. Total bacteria are counted as number predicted with the assistance of a spectrophotometer.

**Results and Discussion**

In this study, the results demonstrated that the all types of commercial bleaching solutions (hypochlorites) have anti-bacterial influence against two species of bacteria (*Staph. aureus* and *E.coli*), and the sensitivity of types bacteria were gradually increased with increasing of bleaching solutions concentrations exception of (Oroplus 50mg/ml) in E.Coli gives little sensitivity about the concentration that has before (shoof 40mg/dl)(Table.2). Previous study demonstrated that investigation in vitro the antimicrobial activity of sodium hypochlorite NaOCl at different concentrations can be used as irrigating solutions owing to their antimicrobial
properties\textsuperscript{15}.

In this research, E. coli and Staphylococcus aureus isolates displayed resistance to hypochlorite solutions\textsuperscript{16} recorded that bacteria completely isolated from contaminated disinfectant solutions and antiseptics display increased resistance to widely used antibiotics, provided that bacteria have the ability to share strong markers and, as resistance arises for one agent, cross-resistance.

The result in (fig1, 2 and table.2) explained that the hypochlorites have been more successful against Staphylococcus aureus than the E.coli confirmed with this finding\textsuperscript{17} especially with Alwazir solution as it gave the high significant efficiency compared with other solutions. The outcomes are agreement with other studies which are indicate that Gram’s negative bacteria are less sensitive to antiseptics and disinfectants. The final result was accepted\textsuperscript{18}, whereby the complicated cell layer, and their outer membrane functions as a permeability shield for restricting or blocking entry of different chemicals\textsuperscript{19}.

The fast reduction rate in the 30S reaction showed the immediate efficiency of Hypochlorites\textsuperscript{20}. The oxidizing mechanism can explain the immediate killing of bleach\textsuperscript{21}. The bleach became bactericidal for vegetative species quickly, which noticed identical tests. After five minutes of exposure to this disinfectant, the 10\% bleach concentration kills all tested bacteria. This contributes primarily to the bleach sterilization mechanism triggered by the oxidation reactions when bleach is mixed with water which lead organisms to be totally destroyed. In addition, four blanching results of the two study bacteria (E. coli and Pseudomonas aeruginosa) was (Fas 50 percent Oroplus 50 \% Shoof 40 \% Alwazuir 30\% Lamaoa 25 \% respectively) For both the two bacteria studied (E.coli and Staphylococcus arueus) the efficacy of culturing pattern was specific and from the analysis Fas 50 \% and Oroplus50 \% had the strongest efficacy against the bacteria studied following by Shoof 40 \% Alwazir 30 \% Lamaoa 25 \% had less efficacy against the bacterial measured\textsuperscript{18}. Hypochlorite concentrations (mg/ml) inhibition zone (mm) Oroplus, Shoof, Alwazir and Lamaoa concentrations indicate a high efficiency against two bacteria studied used in this research, and findings obtained have been confirmed with (Gaonkar \textit{et al.},2006)\textsuperscript{22}.

Fig (1): The effect of bleaching solutions on E.coli bacteria
Table (2): Zone of inhibitions for different concentration bleaching solutions on *E.Coli* and *Staph aureus* bacteria.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
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<tr>
<td><strong>Fas</strong></td>
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<td>50</td>
<td>22</td>
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<tr>
<td>Oroplus 50</td>
<td>17</td>
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<tr>
<td>Shoof 40</td>
<td>19</td>
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<tr>
<td>Alwazir 30</td>
<td>12</td>
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<td>Lamo 25</td>
<td>9</td>
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<tr>
<td>Control D.W</td>
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**E.coli**

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<th>Concentration (mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
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**Staph aureus**

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<th>Concentration (mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
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**Conclusion**

Hypochlorite solutions are strong oxidizing agents, extremely useful chemical used as a disinfectant. The study included five species of hypochlorite solutions widely used as commercial bleaching solutions (*Shoof*, *Oroplus*, *Alwazir*, *Lamo* and *Fas*) and checked their efficiency on bacteria (*E.coli* and *Staph. Aureus*). All types of commercial bleaching solutions (hypochlorites) have anti-bacterial influence against two species of bacteria and bacterial sensitivity was progressively increased with the exception of concentrations of bleaching solution in *Oroplus* 50mg/ml in *E.Coli* which gives little sensitivity about the concentration that has before, the results explained that all the bleaching solutions have been more successful against *Staphylococcus aureus* than the *E.coli*, and the outcomes enhance in agreement with other studies which are indicated that Gram’s negative bacteria are less sensitive to antiseptics and disinfectants. Finally the current study suggested and encouraged to use hypochlorite solutions as antiseptics and disinfectants for killing species of bacteria.

**Acknowledgment:** The author’s gratefully thankful Al-Maarif University College for supporting this work, and we would like to thank general Heet Hospital for technical support and for the supply preparation of isolated bacteria for fulfilled this research.

**Conflaict of Interests:** There are no conflict of interest.

**Ethics and Consent:** Taken from Al-Maarif University College
Funding: Nil.

References


Detection of the Some Dominant Aerobic Microorganisms in Burn Injury and Testing their Susceptibility for Different Antibiotics in Najaf

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Abstract

Burn injury and wound is a pig problem that assists the pathogens to grow and cause infection. 149 burn injury swabs were taken from different site of the patients’ body ages (11-53 years old). All samples were tested to isolate the significant microorganisms that found in burn injury. Antibiotics test was performed by using disc diffusion way that stated by Kirby-Baucer. Results appeared that the most frequent pathogen in burn injury was \textit{Pseudomonas aeruginosa} 33(22.14%) and followed by \textit{E.coli} 31(20.8%), \textit{Staphylococcus aureus} 28(18.79%), \textit{Proteus vulgaris} 26(17.48%), Coagulase-negative \textit{Staphylococci} 12(8.05%), \textit{Proteus mirabilis} 10(6.71%), \textit{Klebsiella pneumonia} 5(3.35%) and \textit{Candida spp.} 4(2.68%) respectively. Antibiotic test showed that \textit{Staphylococcus aureus} was a high resistance against Gentamycin and ciprofloxacin, but it was sensitive to Vancomycin and Imipenem. Coagulase negative \textit{Staphylococci} showed a resistance to Gentamycin, Ciprofloxacin and Ceftazidime, but it was sensitive to Amikacin, Oxacillin, Cefotaxim, Vancomycin, Imipenem and Cefepime. \textit{P. aeruginosa} showed resistance against Cefotaxime, Aztreonam, Gentamcin and Ceftazidime. Whilst it showed a high sensitivity against Imipenem, Cefepime and Tobramycin. \textit{E.coli} showed a high resistance against Ceftriaxone, Ciprofloxacin and Cephalothin. whilst it showed a high sensitivity against Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. \textit{Proteus vulgaris} showed a high resistance against Ceftriaxone, Coagulase negative \textit{Staphylococci} and Cephalothin. whilst it showed a high sensitivity against Ceftriaxone, Amikacin, Cefotaxim, Ticarcillin/clavulanic acid and Ceftazidime. \textit{Proteus mirabilis} showed resistance against Amikacin, Ciprofloxacin, Ceftriaxone, Gentamcin and Cephalothin. Whilst it showed a high sensitivity against Ceftriaxone, Cefazidime, Amikacin, Cephalothin and Gentamicin. whilst it showed a high sensitivity against Cefotaxime, Aztreonam, Ticarcillin/clavulanic acid and Imipenem. \textit{Klebsiella pneumonia} showed resistance against Ciprofloxacin, Ceftazidime, Cefotaxime and Aztreonam. whilst it showed a high sensitivity against Ceftriaxone, Amikacin, Gentamicin, Cephalothin, Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. The current study was aimed to determine the most frequent pathogens in burn injury and test their antibiotics susceptibility in hospitalize patients in Najaf city.

Keywords: Burn injury, Burn infection, Antibiotics.

Introduction

Burn infection considered one of the most medical issue in the world[1], there are many reasons could lead for it, such as heat, chemical agent, electricity… etc. The big problem in burn is the infection when the skin is destroyed, many pathogens and opportunistic microbes are initiated the infection[2] and this lead to sepsis especially in the modern countries[3], and this is the cause of high morbidity and mortality in hospitalized burn patients[4,5]. Burn injury considered a harmful form of trauma so, the patients with burn injury must take essential care to prevent mortality and morbidity[6]. The term of multi-drug resistant Gram-positive bacteria and Gram-negative bacteria is used to the most microorganisms linked to injuries infection,
according to many studies, these microorganisms include *Staphylococcus aureus* which is found in 20–40% and *Pseudomonas aeruginosa* which is found in 5–15% and other microorganisms like *Escherichia coli*, *Klebsiella pneumonia* and *Acinetobacter* spp. consider as a nosocomial pathogens. Topical antibacterial reduce bacteria growth but not prevent growth other potentially invasive bacteria and fungi. These are contaminated the wound patients and their source is initiated from gastrointestinal or upper respiratory tract or the hospital environment itself. After wounds contamination and adherence, these microbes begin to penetrate the viable tissue and invasive it, invasiveness of microbes is depend on the size of local wound and who the patients’ health are, some patients have immunosuppression state. If sub-eschar tissue is colonized, disseminated infection is likely to happen, and Some microorganisms are changed with time, some of them become so aggressive and other microorganism lose it ability to cause infection and this depend on the host immunity. New microorganisms are arrived to the burn ward with new patients. These organisms live with resident flora of the burn for period of time so, they acquire new feature like antibiotics resistance. Presentation of new topical agents and systemic antibiotics influence the flora of the wound could be interested in future to prevent the mortality and morbidity by opportunistic flora.

**Materials and Methods**

**Population in study**

The current study included 149 samples (112 males and 39 females) which collected from patients with burn injury in different sites by assistance of physician or the team care in the patient’s room during the period 5 March 2020 to 12 August 2020 from the burn center in Alsadr hospital in Najaf city, Iraq. the patients in this study didn’t have any chronic diseases or any other infection, the patients ages were 11 to 53 years old. all patient were agreed to cooperate and participate in this study in order to provide service to the community.

**Sample collection**

When the samples were taken from patients under complete aseptic conditions by sterile cotton swabs with sterile normal saline, they put in to brain heart infusion broth (Himedia-India) and they stayed no longer than one hour in the broth (to prevent the growth of contaminated microorganism especially *Aspergillus* spp.), and directly transported to the medical microbiology lab in Altoosi University College and then cultured in to different media (Himedia-India) for microbiology diagnosis that was achieved according to Macfaddin, and Forbes et al.

**Identification of aerobic microorganism**

For each sample in brain heart infusion broth, three petri dishes with Nutrient agar Blood agar, Maccokey, agar mannitol salt agar and Sabouraud dextrose agar (with chloramphenicol). All agars were incubated at 37°C for 24-48h. Subculture for single colonies were achieved to obtain pure colony for each microorganism.

**Antibiotics Test**

The method of antibiotics test was performed by using disc diffusion way that stated by Kirby-Baucer. The pure colony for each bacteria was streaked like mat onto Muellur Hinton agar (Himedia-India). For gram positive bacteria, thirteen different antibiotics disc were used which provided from Oxoid-USA, these antibiotics were as follow: Amoxicillin/Clavulanic acid (20/10 μg), Gentamicin (10 μg), Amikacin (30 μg), Oxacillin (1 μg), Ciprofloxacin (1 μg), Ceftriaxone (30 μg), Ceftazidime (30μg), Cefotaxim (30μg), Cephalothin (30μg), Ticarcillin/clavulanic acid (75/10μg), Vancomycin (30μg), Imipenem (50μg) and Cefepime (10μg). While for gram negative bacteria, twelve different antibiotics disc were used which provided from Oxoid-USA, these antibiotics were as follow: Ciprofloxacin (5μg), Ceftriaxone (30μg), Ceftazidime (30μg), Amikacin (30μg), Gentamicin (10μg), Cefotaxime (30μg), Aztreonam (30μg), Cephalothin (30μg), Ticarcillin/clavulanic acid (75/10μg), Imipenem (10μg), Cefepime (10μg) and Tobramycin (10μg). The sensitivity and resistance of bacteria was determined based on the inhibition zone that form on Muellur Hinton agar after
18-24h from culture. The inhibition zone that form was compared with CLSI 2017\textsuperscript{[14]}.

**Statistical Analysis**

The percentages of the number of was achieved by SPSS version 17 windows7.

**Results**

**Identification of Microorganisms in burn injury**

The present study included 149 burn injury patients. Eight different microorganisms were isolated in this study. Forty (26.84\%) patients swabs were Gram positive bacteria (*Staphylococcus aureus* (18.79\%) and *Coagulase-negative Staphylococci* (8.05\%)), while fifty four (70.48\%) patients swabs were gram negative bacteria (*Pseudomonas aeruginosa* (22.14\%), *E.coli* (20.8\%), *Proteus vulgaris* (17.44\%), *Proteus mirabilis* (6.71\%) and *Klebsiella pneumoniae* (3.35\%). *Candida spp.* was isolated in four (2.68\%) swabs patients. Table 1.

**Table 1. the most frequent microorganisms isolated from burn injury patients**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Frequency N=149</th>
<th>% Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>33</td>
<td>22.14</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>31</td>
<td>20.8</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>28</td>
<td>18.79</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>26</td>
<td>17.48</td>
</tr>
<tr>
<td><em>Coagulase-negative Staphylococci</em></td>
<td>12</td>
<td>8.05</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>10</td>
<td>6.71</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>5</td>
<td>3.35</td>
</tr>
<tr>
<td><em>Candida spp.</em></td>
<td>4</td>
<td>2.68</td>
</tr>
</tbody>
</table>

Antibiotics susceptibility test

**Gram positive bacteria**

*Staphylococcus aureus* Showed a high resistance against Gentamycin and ciprofloxacin, but it was a high sensitivity against Vancomycin and Imipenem. whilst it was had a mild resistance to *Cephalothin* and Amikacin. Table 2.

Coagulase negative *Staphylococci* showed a mild resistance against Gentamicin, Ciprofloxacin and Ceftazidime. but it was a high sensitivity against Amikacin, Oxacillin, Cefotaxim, Vancomycin, Imipenem and Cefepime. Whilst it was had a mild resistance to Gentamicin, Ciprofloxacin, Ceftazidime, table 2.
Table 2. Antibiotics test for Gram positive bacteria isolated from burn injury and their concentrations.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance of Staphylococcus aureus N=28 (18.79%)</th>
<th>Resistance of Coagulase negative Staphylococci N=12 (8.05%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin/Clavulanic acid (20/10 μg)</td>
<td>5 (17.8%)</td>
<td>1 (8.33%)</td>
</tr>
<tr>
<td>Gentamicin (10 μg)</td>
<td>11 (39.28%)</td>
<td>2 (16.66%)</td>
</tr>
<tr>
<td>Amikacin (30 μg)</td>
<td>4 (14.28%)</td>
<td>S*</td>
</tr>
<tr>
<td>Oxacillin (1 μg)</td>
<td>1 (3.57%)</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin (1 μg)</td>
<td>7 (25%)</td>
<td>2 (16.66%)</td>
</tr>
<tr>
<td>Ceftriaxone (30 μg)</td>
<td>2 (7.14%)</td>
<td>1 (8.33%)</td>
</tr>
<tr>
<td>Ceftazidime (30μg)</td>
<td>2 (7.14%)</td>
<td>2 (16.66%)</td>
</tr>
<tr>
<td>Cefotaxim (30μg)</td>
<td>1 (3.57%)</td>
<td>S</td>
</tr>
<tr>
<td>Cephalothin (30 μg)</td>
<td>5 (17.85%)</td>
<td>1 (8.33%)</td>
</tr>
<tr>
<td>Ticarcillin/clavulanic acid (75/10μg)</td>
<td>2 (7.14%)</td>
<td>S</td>
</tr>
<tr>
<td>Vancomycin (30 μg)</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem (50 μg)</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefepime (10 μg)</td>
<td>2 (7.14%)</td>
<td>S</td>
</tr>
</tbody>
</table>

* S= Sensitive

Gram Negative Bacteria

*Pseudomonas aeruginosa* showed resistance against Cefotaxime, *Aztreonam*, Gentamicin and Ceftazidime, but it showed a high sensitivity against Imipenem, Cefepime and Tobramycin. Whilst it was had a mild resistance to Amikacin, Ceftriaxone, *Cephalothin and Ciprofloxacin* Table 3.

*E.coli* showed a high resistance against Ceftriaxone, Ciprofloxacin and Cephalothin. But it showed a high sensitivity against Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. Whilst it was had a mild resistance to Amikacin, Gentamicin, Cefotaxime, Aztreonam, and Ceftazidime. Table 3.

*Proteus vulgaris* showed a high resistance against Ceftriaxone, Ceftazidime, Amikacin, *Cephalothin* and Gentamicin. But it showed a high sensitivity against Cefotaxime, *Aztreonam, Ticarcillin/clavulanic acid* and Imipenem. Whilst it was had a mild resistance to *Ciprofloxacin and Tobramycin* Table 3.

*Proteus mirabilis* showed resistance against Amikacin, Ciprofloxacin, Ceftriaxone, *Gentamicin* and Cephalothin. But it showed a high sensitivity against Ceftazidime, Cefotaxime, *Aztreonam, Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin.*
Whilst it was had a mild resistance to Amikacin Table 3.

*Klebsiella pneumonia* showed resistance against Ciprofloxacin, Ceftazidime, Cefotaxime and Aztreonam. whilst it showed a high sensitivity against Ceftriaxone, Amikacin, Gentamicin, Cephalothin, Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. Table 3.

**Table 3.** Antibiotics test for Gram Negative bacteria isolated from burn injury and their concentrations.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>P. aeruginosa</em> N=33 (22.14%)</th>
<th><em>E.coli</em> N=31 (20.8%)</th>
<th><em>Proteus vulgaris</em> N=26 (17.44%)</th>
<th><em>Proteus mirabilis</em> N=10 (6.71%)</th>
<th><em>Klebsiella pneumonia</em> N=5 (3.35%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin (5μg)</td>
<td>4 (12.12%)</td>
<td>10 (32.25%)</td>
<td>2 (7.69%)</td>
<td>1 (10%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Ceftriaxone (30μg)</td>
<td>6 (18.18%)</td>
<td>11 (35.48%)</td>
<td>10 (38.46%)</td>
<td>1 (10%)</td>
<td>S</td>
</tr>
<tr>
<td>Ceftazidime (30μg)</td>
<td>7 (21.21%)</td>
<td>4 (12.9%)</td>
<td>10 (38.46%)</td>
<td>S</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Amikacin (30 μg)</td>
<td>6 (18.18%)</td>
<td>6 (19.35%)</td>
<td>9 (34.61%)</td>
<td>2 (20%)</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin (10μg)</td>
<td>7 (21.21%)</td>
<td>6 (19.35%)</td>
<td>7 (26.92%)</td>
<td>1 (10%)</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxime (30μg)</td>
<td>9 (27.27%)</td>
<td>5 (16.12%)</td>
<td>S</td>
<td>S</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Aztreonam (30μg)</td>
<td>8 (24.24%)</td>
<td>5 (16.12%)</td>
<td>S</td>
<td>S</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Cephalothin (30μg)</td>
<td>5 (15.15%)</td>
<td>7 (22.58%)</td>
<td>8 (30.76%)</td>
<td>1 (10%)</td>
<td>S</td>
</tr>
<tr>
<td>Ticarcillin/clavulanic acid (75/10μg)</td>
<td>1 (3.03%)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem (10μg)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefepime (10μg)</td>
<td>S</td>
<td>S</td>
<td>1 (3.84%)</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tobramycin 10μg</td>
<td>S</td>
<td>S</td>
<td>2 (7.69%)</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

**Discussion**

Burns infection death are the most notified event that occur between admitted hospitalize patients which represent over 50% of burn deaths. Although many burns centers gave specialize medical care for patients, but the nosocomial infections still the big problem for care teams[18]. In the current study, the most frequent pathogen in burn infection was *P. aeruginosa* (22.14%) in Najaf city, followed by *E.coli* (20.8%), *Staphylococcus aureus* (18.79%), *Proteus vulgaris* (17.44%), *Coagulase-negative Staphylococci* (8.05%),
Proteus mirabilis (6.71%) and Klebsiella pneumonia (3.35%). These results were in agreement with finding stated by Aljanaby et al, they found in their a three years cross-sectional study that the most frequent pathogen was P. aeruginosa (27.6%)\textsuperscript{[19]}. This could be belong to the fact that P. aeruginosa is one of the most significant microbes which is distributed in the nature. P. aeruginosa has ability to produce infection because the high rate of virulence and antimicrobial resistance\textsuperscript{[20,21]} But the current study disagree with the finding that stated by Al-Kanaany, she find the majority isolated organism in burn infection was Staphylococcus and followed by Pseudomonas. this difference is not significant because in the current study S. aureus was isolated as a third frequent pathogens in burn infection. although Both P. aeruginosa and S. aureus have high diffusion rate and consider as a nosocomial pathogens in admitted Hospitalize patients. In this study E. coli, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumonia and Candida were isolated and this result are in agreement with previous studies\textsuperscript{[22,23]}. present of these bacteria may belong to environmental factor that contaminate the wounds, some patients could take these bacteria from hospital. Modern study showed that there was a relation-ship between admitted hospitalize patient for long time and the prevalence of pathogenic bacteria in burn\textsuperscript{[19]}.

Results showed the most isolated bacteria which has multi-antibiotics, resistance is P. aeruginosa and followed by S. aureus, E.coli and Proteus vulgaris. this either could belong to their ability to acquire the genes that responsible for drug resistance from other bacteria in the environment though plasmid or other vector by conjugation or any methods of gene transfer, or belong to ability of Gram negative bacteria especially P. aeruginosa to form biofilm that protect bacteria from the effect of host immunity and antibiotics\textsuperscript{[24-26]}. P. aeruginosa is one of the most important pathogens causing different infections such as bacteremia and burn infection\textsuperscript{[27]}. P. aeruginosa alone has more than 70% mortality of Burn infection\textsuperscript{[28]}.

Conclusions

From the presented study, the author conclude that the most dominant pathogens in burn infection was Pseudomonas aeruginosa and followed by Staphylococcus aureus, Proteus vulgaris, Coagulase-negative Staphylococci, Proteus mirabilis, Klebsiella pneumonia and Candida spp. We also conclude that Imipenem (10μg) is an excellent choice for burn infection as a first choice, it has 100% against all the pathogens that isolated in the current study, while Ticarcillin/ clavulanic acid (75/10μg) and Cefepime (10μg) consider the second choice.

Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearance: Taken from Altoosi University College committee.

Acknowledgements: The author acknowledges Alsadr hospital-burns center teams for their assistance in sample collection. The author is dedicated this work to the soul of his best friend Assist. Prof. Dr. Raad Alharmoosh, Co-dean of Altoosi college University.

References


Effect of Dentifrices with Different Abrasives on the Surface Roughness of a Nano Composite Resins materials

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Abstract

Background: to evaluate the effect of different dentifrices on the surface roughness of two composite resins (nanofilled-based and nanoceramic – based composite resins). Materials and methods: Forty specimens (diameter 12 mm and height of 2mm) prepared from different composite resin materials: Z350 (nanofilled composite, and Ceram-X (nanoceramic). they were subjected to brushing simulation equivalent to the period of 1 year. The groups assessed were a control group brushed with distilled water (G1), Opalescence whitening toothpaste (G2), Colgate sensitive pro-relief (G3) and Biomed Charcoal Toothpaste (G4). The initial and final roughness of each group was tested by surface roughness tester. The results were statistically analyzed using ANOVA and Tukey test at 0.05 significance level. Results: the surface roughness of the two tested composites brushed with the tested dentifrices was statistically higher than the roughness found in control group. Comparison among the three types of dentifrices showed that there was a statistically high significant difference in the surface roughness among all subgroups. Charcoal Toothpaste showed the highest surface roughness increase. Conclusion: Whitening dentifrices increase the surface roughness of dental composite thereby compromising its durability. Changes in composite depended on the material itself and the dentifrices used.

Key words: Abrasive, Composite resin, Dentifrices, Surfaces roughness, Whitening.

Introduction

Restorative materials have a lot of refinement in the mechanical, physical, chemical properties and esthetics since their introduction (1). The composites produced with the aid of nanotechnology became popular as they offer longevity and better esthetic (2). The recent advances in nanotechnology yielded nano-filled and nano-ceramic composite resin materials (3). Nanofill composites are composted of nanoclusters and nanosilica particles, the nanocluster fillers are collections of particles of nano-size and act as one unit to achieve higher filler strength and loading up to 80% of the resin matrix’s total weight (3, 4). The organically modified nanoceramic composite was developed by combining the nanotechnology and ormocer technology. This composite contains methacrylate-modified, silicon dioxide–containing nanofiller of 1.1–1.5 μm in size that account for 76% of total weight and resin matrix that is replaced by a matrix full of highly dispersed methacrylate-modified polystyloxane particles (2, 5).

The dental materials’ surface texture has a major influence on plaque accumulation, wear, discoloration and the aesthetic appearance of direct and indirect restorations (6). The surface roughness can wear out the opposite tooth enamel and reduce patient satisfaction and comfort (7). Tooth brushing with the aid of dentifrices decreases dental caries but might damage the surface of resin composite restorations, making it rougher, and more liable to staining and plaque accumulations (8). Different dentifrices are available in the markets to provide fast, easy and low-cost whitening effect (9).

The wear caused by tooth brushing depend mainly on tooth brushing habits, the quality of the toothbrush (hard, medium, or soft), and the dentifrice abrasive used (10). The abrasive particles are insoluble minerals designed to remove microorganisms, stains and disorganize the bacterial biofilm giving a whitened appearance (11). In dentifrices, the most widely used abrasives are hydrated silica, calcium carbonate, calcium phosphate, sodium bicarbonate and calcium pyrophosphate (12).
Other abrasives include tricalcium phosphate, hydrated alumina, calcium sulfate and sodium metaphosphate (11). Most whitening dentifrices act on extrinsic stain removal as they do not contain bleaching agents (13).

Activated charcoal dentifrices have the capacity of adsorbing pigments, chromophores and stains responsible for the color change of teeth (14). However, charcoal’s shape, composition and sizes of its particles could be abrasive and may increase the surface roughness of composite resin (15). Long-term tooth brushing with abrasive toothpastes, can compromise the esthetic appearance and hasten the degradation of the restoration (16).

This study measures the surface roughness of a nanofilled and nanoceramic composite resin after brushing with different types of dentifrices. The null hypothesis is that the dentifrices do not change the surface roughness of restorative material.

**Materials and Methods**

The composites chosen for this study (Table 1) were the following: Z350 (nanofilled composite, 3M ESPE, St. Paul, MN, USA) and The Ceram-X (nanoceramic composite, Dentsply DeTrey, Konstanz, Germany). Three brands of dentifrices containing different compositions were used: Opalescence whitening toothpaste® R, Colgate sensitive pro-relief® and Biomed Charcoal Toothpaste.

**Specimens’ preparation**

Forty specimens (disks) were made for each tested composite (2 X 12 mm). The specimens were photoactivated using a light curing device 1200Mw/cm² Blue curing light (Guilin woodpecker medical instrument, China) for 20 seconds of exposure time to both top and bottom surfaces, respectively according to the manufacturer’s instructions. Samples were polished with rubber abrasive points (Politip-P, Ivoclar Vivadent Inc., US) and stored in distilled water at 37°C for 24 hours, to stimulate clinical situation (4).

**Specimens’ Grouping**

Specimens made from tested composite were randomly divided into four groups (n = 10) as follows: G1 – brushed with distilled water (control group); G2 – brushed with Opalescence whitening toothpaste® R; G3 – brushed with Colgate sensitive pro-relief®; G4 – brushed with Biomed Charcoal Toothpaste. The composition of testing toothpastes presented in Table 2.

**Brushing**

A specially designed brushing apparatus was designed by the Department of Conservative and Aesthetic Dentistry, College of Dentistry, University of Baghdad. The brushing apparatus was set to run a horizontal course of 3.8 cm at a speed of 356 RPM, and a load of 200 g (17). For all groups, the samples were fixed and brushed with corresponding dentifrice for 60 minutes, simulating to 1 year of brushing. Toothpaste was mixed with distilled water (ratio 1:1) (1, 2, 9). After tooth brushing, to avoid interference with the results, specimens were washed with water to remove all the remnants. Similar toothbrushes (Signal, Spain) fixed to the brushing apparatus supports and adjusted so that the bristles would come into contact with the specimens.

**Surface Roughness Measurements**

A profilometer (Hand-Held Roughness Tester, TR200, Time Group Inc. China) were used to determine the initial surface roughness (prebrushing) and the post brushing surface roughness. Specimens measured by the profilometer in different three areas, then the average value registered as the mean Ra of the specimen. The mean Ra values were automatically measured by the profilometer.
Table 1: composition and manufacturers of tested composite resins

<table>
<thead>
<tr>
<th>Composite resin</th>
<th>Composition Particle</th>
<th>size</th>
<th>Load percentage</th>
<th>Manufacturer</th>
</tr>
</thead>
</table>
| Z350 (Nanocomposite) | Matrix: Bis-GMA, Bis-EMA, UDMA, TEGDMA  
Filler: zirconia and silica | 20nm silica filler  
4-11nm zirconia filler | 78.5wt% (63.3 vol%) | 3 M ESPE, Sumaré, SP, Brazil |
| Ceram-x: Nanoceramic Resin composite | Matrix: Methacrylate modified polysiloxane, dimethacrylate resin  
Filler: Barium-aluminum-borosilicate glass, methacrylate functionalized silicon dioxide (nano filler) | (0.04-4 um) 10 nm | 76 wt% (57vol%) | Dentsply/Caulk, Milford DE, USA |

Table 2: Description of dentifrice used in this study

<table>
<thead>
<tr>
<th>Dentifrice</th>
<th>Composition</th>
<th>Abrasive product</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opalescence whitening toothpaste²</td>
<td>Sodium Fluoride 0.25%w/w (Anticavity) Glycerin, Water (aqua), Silica, Sorbitol, Xylitol, Flavor (aroma), Poloxamer, Sodium Lauryl Sulfate, Carbomer, FD&amp;C Blue#1 (CI 42090), FD&amp;C Yellow#5 (CI 19140), Sodium Benzoate, Sodium Hydroxide, Sparkle (CI 77019, CI 77891), Sucralose, Xanthan Gum.</td>
<td>Silica, Mica (CI 77019), titanium dioxide (CI 77891)</td>
<td>Ultradent products, inc.south Jordan, UT84095,USA</td>
</tr>
</tbody>
</table>
| Colgate® Snesative PRO-Relief™ whitening | Sodium Monofluorophosphate 1.1%/w (1450 ppm F)  
Calcium Carbonate, Aqua, Sorbitol, Arginine, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Cellulose Gum, Sodium Bicarbonate, Tetrasodium Pyrophosphate, Sodium Saccharin  
Benzyl Alcohol, Sodium Saccharin, Xanthan Gum, LimoneneCI 77891 | Calcium Carbonate Sodium Bicarbonate titanium dioxide (CI 77891) | Colgate-palmolive manufacturing (Poland)Sp.-z.o.o., Al. Colgate 2,58-100 Swidnica, Poland . |
| Biomed Charcoal Toothpaste | Aqua, Hydrogenated Starch Hydrolysate, Dicalcium Phosphate Dihydrate, Hydrated Silica, Glycerin, Sodium Coco-Sulfate, Cellulose Gum, Aroma, Calcium Hydroxyapatite, Zinc Citrate, Tetrasodium Glutamate Diacetate, Benzyl Alcohol, Sodium Bicarbonate, Xanthan Gum, Menthyl Lactate, Xylitol, Carbon Black, Charcoal Powder, Cymbopogon Flexuosus Herb Oil, Mentha Piperita Oil, Ananas Sativus Fruit Extract, Maltodextrin, Cinnamomum Camphora Bark Oil, Cedrus Atlantica Bark Oil, Betula Alba Leaf Extract, Plantago Major Leaf Extract, Arginine, Sodium Hydroxide, Sodium Benzoate, Potassium Sorbate, Limonene, Citral | Hydrated Silica, Sodium Bicarbonate 3 types of charcoal (bamboo, activated charcoal and wood charcoal) | Biomedglobal,russia |
Result

Descriptive statistics: Means, minimum, maximum and standard deviation of composite roughness values were listed in Table 3. According to the table there was an increase in the means of roughness values for tested composite after brushing with tested dentifrices, also the Z350 had the lowest roughness values and Ceram x had the highest value.

Inferential statistics: ANOVA test used for all groups showed that there is a high significant difference ($p<0.001$) in surface roughness values among the groups for each composite resin after brushing with distilled water or different dentifrices which show a high significant difference in surface roughness Ra values among the tested composite materials as shown in Table 3.

Table 3: Descriptive and ANOVA test among the three tested composite

For intragroup comparison, Tureky test for all groups were done which showed highly significant increases in surface roughness value Ra of the two tested composite ($p>0.001$) after brushing with distilled water and Opalescence whitening toothpaste and non-significant differences ($p<0.05$) in surface roughness value after brushing with Colgate sensitive pro-relief and Biomed Charcoal Toothpaste as shown in Table (4).
Table 4: Tukey test for surface roughness of the tested composite

<table>
<thead>
<tr>
<th>Tukey HSD Dependent Variable</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>P-Value</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>G2</td>
<td>-.0270*</td>
<td>.00688</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>-.1960*</td>
<td>.00688</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>-.2160*</td>
<td>.00688</td>
<td>.000</td>
</tr>
<tr>
<td>G2</td>
<td>G3</td>
<td>-.1690*</td>
<td>.00688</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>-.1890*</td>
<td>.00688</td>
<td>.000</td>
</tr>
<tr>
<td>G3</td>
<td>G4</td>
<td>-.0200*</td>
<td>.00688</td>
<td>.030</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

Another analysis was the difference after brushing with the tested Dentifrices equation: Before-after = ∆Ra*

The data revealed that the lower surface roughness difference was with the use of distilled water, followed by Opalescence whitening toothpaste\textsuperscript{R}, Colgate sensitive pro-relief and Biomed Charcoal Toothpaste as shown in table 5.

Table 5: ∆Ra difference between after and before surface roughness of the tested composite.

<table>
<thead>
<tr>
<th></th>
<th>before</th>
<th>after</th>
<th>∆Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>z350 Distilled water</td>
<td>1.48</td>
<td>1.56</td>
<td>0.08</td>
</tr>
<tr>
<td>Opalescence</td>
<td>1.5</td>
<td>1.61</td>
<td>0.11</td>
</tr>
<tr>
<td>Colgate</td>
<td>1.55</td>
<td>1.83</td>
<td>0.28</td>
</tr>
<tr>
<td>Charcoal</td>
<td>1.6</td>
<td>1.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Cont.. Table 5: $\Delta$Ra difference between after and before surface roughness of the tested composite.

<table>
<thead>
<tr>
<th>Effect of the dentifrice</th>
<th>Distilled water</th>
<th>Opalescence</th>
<th>Colgate</th>
<th>Charcoal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceram x</td>
<td>3.07</td>
<td>3.15</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>3.21</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.15</td>
<td>3.45</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>3.53</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Direct composite resins are always exposed to chemical and mechanical challenges that can alter the material properties such as when associated with long term brushing, specially when the toothpaste contain hard abrasive particles, so the composite resin will degrade and compromise giving opposite results from what was expected from the whiting dentifrices (11, 12). Different dentifrices with different compositions have been developed to improve the affectivity of cleaning and whitening of teeth. Few of them have hydrogen peroxide (1%) in its formulation which is the active principle of bleaching products used in the dental office because it can cause irritation of the gingival tissues and lead to dentin hypersensitivity (18). Other substances used by cosmetic industries in whitening dentifrices are optical pigments such as titanium dioxide and blue covarine, which can color the enamel surface white, more than the effect in removing, stains (19).

Regardless of the addition of hydrogen peroxide or pigments to whitening dentifrice, abrasive substances are often present in their components to increase the friction during tooth brushing, which can jeopardize resin composite without contributing to whiten the teeth. So the purpose of this study was to evaluate the abrasiveness of three different dentifrices on the surface roughness of nano-manufactured composite resins after simulating one year of tooth brushing.

This study used parameters accommodated the clinical situation, such as the amount of toothpaste (ratio of 1:1), brushing load (200 gr), and brushing time (17). Brushing time was calculated by the assumption that brushing for two minutes (120 seconds) twice daily, divided on an average of 24 teeth result in 10 seconds of brushing time for every tooth per day, brushing for one year was assumed to take 3650 seconds (60 min) (20).

The results showed that, the surface roughness was significantly affected by the dentifrice factor, so rejecting the null hypothesis. This finding agrees with other studies that showed a correlation between tooth brushing with the use of abrasive dentifrice and the increase in the surface roughness of composite (11, 12, 21).

A study conducted by da Oliveira et al. (22), assessed the effect of simulated tooth brushing with different whitening dentifrices on the surface roughness of different composite resins which found a significant difference in the result for the dentifrice factor, supporting the findings of our study.

The increased Ra value may be caused by the mechanical action of the brushing and the abrasive material contained in the dentifrice (23). The mechanical factor was controlled accordingly in all groups. There was a statistical difference in the roughness values after the brushing challenge (Table 3) regardless of the toothpaste type even with brushing with distilled water (no toothpaste).

Friction causes the filler particles to fall out from the matrix, leading to increase in the surface roughness of composite resins this effect becomes even greater if accompanied by abrasive toothpaste (24) (25).

The dentifrices containing silica alone are considered to have low abrasiveness but if combined with calcium carbonate, sodium pyrophosphate, titanium oxide, and sodium phosphate, it is considered a high abrasive dentifrice (26).
Amaral et al. (27), study concluded that sodium bicarbonate is more abrasive than silica or calcium carbonate. This finding is consistent with the results of this study, in which the dentifrice Opalescence has silica as an abrasive agent only, so the surface roughness of it was the lowest. While Colgate® Snesative PRO-Relief™ whitening has calcium carbonate and sodium bicarbonate.

The highest increase in the Ra value occurred in Charcoal toothpaste which in addition to having hydrated silica and Sodium Bicarbonate contains activated charcoal which is too abrasive and can wear away tooth enamel (21).

Effect of composite

Regarding the composite assessed in this study, Z350 and Ceram X were chosen because both of them had nanoparticle fillers in their composition. Resin composites with nanofiller has good aesthetic result and good wear resistance (25). Nanoclusters has been also improved to be better wear resistance as the silane would fill the gaps and voids that observed in microhybrid composites (28).

The composition of the resin matrix, filler particle type or content, matrix/filler interface, degree of polymerization and hardness of the resin composites could affect the surface condition after tooth brushing (29, 30). Other parameters such as the polishing and light-curing method are also of fundamental parameter to the values of surface roughness. However, they were standardized and the two tested materials were submitted to the same parameters as for the polishing type / light-curing method (31). For comparative Filtek Z350, the fillers are composed of zirconia/silica, classified as a nanofilled composite, while Ceram X the fillers are silica and radiopaque barium-aluminum-silicate glass, classified as nanohybrid.

In this study Filtek Z350 presented the smoothest surfaces. Since the filler loading of the nano-filled composites is higher than that of the nano-ceramic composites, and this nanofilled materials have the ability to provide more volume of filler in homogeneous distribution, which enables it to protect organic matrix wear (11).

The nano-ceramic composite resin Ceram x demonstrated significantly greater roughness than the nano-filled composite resins Filtek Z350 Ceram; Ceram X is a nano hybrid containing organically modified ceramic nano particles comprising polysiloxane back bone. These nanoceramic particles can be best described as inorganic–organic hybrid particles where the inorganic siloxane part provides strength and the organic methacrylic part makes the particles compatible and polymerizable with the resin matrix (32). However, this modification of the matrix might cause a larger surface roughness of the Ormocer composite when compared to the conventional materials, due to the characteristic of its organic-inorganic resin matrix (30). Many studies have proposed a correlation between surface roughness and filler size, and that composites contain large filler size tend to be rougher than composites contain smaller filler size(23, 29)This finding agrees with the results of this study, as the Filtek Z350, which incorporates the smallest filler particles (from 0.2 to 1.4 µm), presented the smoothest surfaces than the Ceram X composites (from 0.4 µm up to 4.0 µm) glass filler.

The present study had some limitations, the concentration of abrasive materials in the toothpaste were not mentioned in the product which is very important in determining the abrasiveness of dentifrices, also the brushing was performed with dentifrice diluted in distilled water, but in fact this dilution occurs in saliva and the saliva properties can reduce the abrasive effects on the composite, as the saliva provides a sliding surface attenuating composite wear.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Self–funding

Ethical Clearance: The researchers already have ethical clearance from College of Dentistry, University of Baghdad, Iraq.

References


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Coenzyme Q10 in the Follicular Fluid and Its Relation to Oocyte Maturity, Fertilization Rate, Embryo Grading, and Pregnancy Rate

Alaa Abdulateef Mohammed, Zainab Hassan Al-Khafajy, Ass. Prof Dr. Wasan Adnan Abdullhameed

Abstract

Background: Infertility was define as; a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular unprotected sexual intercourse, or due to an impairment of a person’s capacity to reproduce, either as an individual or with his/her partner. According to the latest definition by the international glossary on infertility and fertility care, regular sexual intercourse is an important determinant for the occurrence of pregnancy.

Aim of the study: To assess the relation between Coenzyme Q10 level in the follicular fluid with oocyte maturity, fertilization rate, embryo grading, and pregnancy rate

Patients and method: A prospective cross sectional study conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University and in fertility center at Al-Sadr general hospital during the period from December 2019 to August 2020. Sixty infertile couples were enrolled in this study; all underwent ICSI cycles

Results: Mean level of Co Q10 in Grade III and IV (0.387 ± 0.54) than that in Grade I and II (0.539 ± 0.65), CoQ10 total in pregnant were 0.79 ± 0.63 and in non-pregnant were 0.381 ± 0.2, A threshold of 0.27 of CoQ10 had a sensitivity of 80.0% and specificity of 67.0 %, PPV was (88.0%), NPV (45%) and accuracy was (74%). ROC curve of CoQ10 for pregnancy prediction

Conclusion: Significant decrease of Co Q10 between in Grade III and IV than that in Grade I and II. CoQ10 total were increase in pregnant women than non-pregnant.

Keyword: CoQ10, pregnant rate, oocyte maturity, fertilization rate, embryo grading.
and females are equally responsible for the causes of infertility, and most of the infertile couples have one of these three major causes including a male factor, ovulatory dysfunction, or tubal peritoneal disease. (3)

If the female partner is 35 year of age or older, evaluation should be initiated after 6 months of unprotected intercourse. Fecundability, or the ability to achieve pregnancy in one menstrual cycle, is a more accurate measurement to evaluate fertility potential. The fecundity rate in a normal couple who has had unprotected intercourse is approximately 20% to 25% for the first 3 months, followed by 15% during the next 9 months. (4)

Assisted Reproductive Technology (ART) is defined as a fertility treatment in which eggs and sperm are handled for the purpose of establishing a pregnancy. (5) Assisted Reproductive Technology (ART) now enables several treatment options, including ovulation induction followed by in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). (6)

Coenzyme Q10 (CoQ10), also known as ubiquinone or ubidecarenone, is a vitamin-like nutrient and lipid-soluble compound. As its name implies, it is ubiquitous and present in all human cells. It is primarily located in the mitochondria and also found in cell membranes and lipoproteins. The primary function of CoQ10 is its role in cellular energy production, where, along the inner mitochondrial membrane, the electron transport chain (ETC) uses CoQ10 as a component in oxidative phosphorylation converting products of metabolism (carbohydrates, fats, and proteins) into energy as ATP. (7)

**Oocyte quality and coenzyme Q10**

In in vitro fertilization cycles, the most important issue was the quality of oocyte. The main factors affecting the oocyte quality are the age of the women and the status of ovarian reserve. The inappropriately age-related decrease in oocyte quality is a main difficulty in the treatment of older patients. The main reason for this decrease was related to an accumulation of point mutations and deletions of mitochondrial deoxyribonucleic ac-id (DNA). (8,9)

**Aim of the Study**

To assess the relation between Coenzyme Q10 level in the follicular fluid with oocyte maturity, fertilization rate, embryo grading, and pregnancy rate

**Patients and Method**

A prospective cross sectional study conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University and in fertility center at Al-Sadr general hospital during the period from December 2019 to August 2020. The study was approved by the local Medical Ethical Committee of the Arab Board council/ Obstetrics and Gynecological department. Sixty infertile couples were enrolle in this study; all underwent ICSI cycles. All selected patient was subjecte to:

- The basic fertility workup of the fertility center that consists of history- taking, physical examination, ovulation detection, evaluation of tubal patency and uterine cavity, and semen analysis.

- Assessment of follicular fluid Coenzyme Q10 level in the day of ova pick up.

**Inclusion criteria:**

- Age 18-40 years old.

- Early follicular phase FSH, LH, cycle day 2 E2, TSH and prolactin (PRL) hormonal level which was done as part of the work up must be within normal.

- Unexplained infertility or tubal function infertility

- Fresh transfer

- Informed consent

- Regular Menstrual cycle

- Normal ovulatory function

- Normal BP, not smoking, not taking any supplements

**Exclusion Criteria:**

- Age >40 years old.

- Abnormal uterine cavity due to polyp myoma, or congenital anomalies, endometriosis.

- Uncontrolled systemic disease as diabetes
mellitus, HT, or uncontrolled endocrinological disorder.

- Women with empty follicle syndrome.
- Women with no fertilized oocyte.
- Couples with semen collection failure at oocyte retrieval day
- Male factor infertility
- Diminished ovarian reserves as assessed by AFC < 5 and AMH < 1.2 ng/dl.

**Statistical Analysis**

Collected data were analyzed using SPSS version 23.0 for windows (SPSS Statistics, IBM, USA) and the results were expressed as mean ± standard deviation (SD). Differences of means within groups were examined by paired sample t-test. P values < 0.05 were considered as statistically significant

**Results**

Pregnancy were happened in 33/60 patients (55%) and 27/60 (45%) were not pregnant (fig 1)

![Fig 1: Pregnancy outcome in the studied group](image)

**Oocytes characteristics in pregnant and non-pregnant ladies**

Table 1, demonstrated the oocyte characteristics of pregnant and non-pregnant ladies. The mean total number of retrieved oocytes in pregnant ladies was 9.90 ±6.34; and that of non-pregnant women was 8.61 ± 3.88 the difference was statistically not significant (P=0.37). However, mean of number of abnormal and ruptured oocytes was significantly lower in women who succeeded to get pregnant when compared to that of women who failed to get pregnant, 1.02 ±0.51 versus 1.48 ±1.06, respectively (P=0.04). There was also no significant difference in mean germinal vesicle oocyte number between both groups, 1.18 ±1.02 versus 1.10 ±0.92, respectively (P=0.7). The difference in mean MI and MII oocyte numbers between both groups were also not significant, 1.41 ±1.34 versus 1.32±1.06 and 6.49 ±3.51 versus 6.01 ±2.38, respectively (P > 0.05). In addition, there was no significant difference in mean number of injected oocytes between pregnant and non-pregnant women, 7.22±4.43 versus 6.51±2.46 (P = 0.47).
Table 1: Oocyte characteristics in the studied group

<table>
<thead>
<tr>
<th></th>
<th>GV</th>
<th>MI</th>
<th>MII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.18 ± 1.02</td>
<td>1.41 ± 1.34</td>
<td>6.49 ± 3.51</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.10 ± 0.92</td>
<td>1.32 ± 1.06</td>
<td>6.01 ± 2.38</td>
</tr>
<tr>
<td>Probability</td>
<td>0.7 NS</td>
<td>0.82 NS</td>
<td>0.5 NS</td>
</tr>
</tbody>
</table>

- n: number of cases; †: Independent samples t-test; NS: not significant at P > 0.05; S: significant at P ≤ 0.05; GV: germinal vesicle; MI: metaphase I oocytes; MII: metaphase II oocytes

Fertilization rate and embryo characteristics in pregnant and non-pregnant women

These characteristics were shown in Table (2). The difference in mean fertilization rate between pregnant and non-pregnant women was statistically not significant, 74.01 ± 21.38 versus 68.91 ± 15.67, respectively (p=0.2). In addition, there was significant difference in mean number of grade I and II embryos between pregnant and non-pregnant groups, 3.87 ± 1.03 versus 2.01 ± 1.09, respectively (P= <0.001). Moreover, there was significant difference in mean number of grade III and IV embryos between pregnant and non-pregnant groups, 0.85 ± 0.61 versus 1.92 ± 0.89, respectively (P= <0.001).

Table 2: Fertilization rate and embryo quality

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pregnant (n=33)</th>
<th>Non pregnant (n=27)</th>
<th>P value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate (%)</td>
<td>74.01 ± 21.38</td>
<td>68.91 ± 15.67</td>
<td>0.2 NS</td>
</tr>
<tr>
<td>Number of GI and GII embryos</td>
<td>3.87 ± 1.03</td>
<td>2.01 ± 1.09</td>
<td>&lt;0.001 S</td>
</tr>
<tr>
<td>Number of GIII and GIV embryos</td>
<td>0.85 ± 0.61</td>
<td>1.92 ± 0.89</td>
<td>&lt;0.001 S</td>
</tr>
</tbody>
</table>

- n: number of cases; †: Independent samples t-test; S: significant at P ≤ 0.05

Coenzyme Q 10 levels in follicular fluid and with pregnancy result

The mean concentration of coenzyme Q 10 in the grade I and II was 0.539 ± 0.65 ng/ml while for grade III and IV was 0.387 ± 0.54. Significant decrease in CoQ10 in grade III and IV than that in grade I and II (P=0.03) (Table 3).
Table 3: The association between coenzyme Q 10 levels in follicular fluid with embryo quality

<table>
<thead>
<tr>
<th>Embryo quality</th>
<th>CoQ10 (ng/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I and II</td>
<td>0.539 ± 0.65</td>
<td>0.03</td>
</tr>
<tr>
<td>Grade III and IV</td>
<td>0.387 ± 0.54</td>
<td></td>
</tr>
</tbody>
</table>

Coenzyme Q 10 levels in follicular fluid with pregnancy results

The mean level of Coenzyme Q 10 in pregnant women was 0.79 ± 0.63 and in non-pregnant women was 0.381 ± 0.21 with significant increase in pregnant women (P=0.002) (Table 4).

The mean level of Coenzyme Q 10 in women within the age < 35 years old was 0.66± 0.23 and in women with age ≥ 35 years was 0.521 ± 0.21 with significant difference were found (P=0.04) (Table 5).

Table 4: The differences between coenzyme Q 10 levels in follicular fluid with pregnancy results of the studied group

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n=33)</th>
<th>Non-pregnant (n=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10 (ng/ml)</td>
<td>0.79 ± 0.63</td>
<td>0.381 ± 0.21</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 5: The differences between coenzyme Q 10 levels in follicular fluid with age of the studied group

<table>
<thead>
<tr>
<th></th>
<th>&lt;35 years (n=45)</th>
<th>≥ 35 years (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10 (ng/ml)</td>
<td>0.66± 0.23</td>
<td>0.521 ± 0.21</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The area under curve (AUC) of serum levels of CoQ10 for expectation of pregnancy was (0.80) (p = 0.002, 95% confidence interval 0.63–0.91). A threshold of 0.27 of CoQ10 had a sensitivity of 80.0% and specificity of 67.0 %, PPV was (88.0%), NPV (45%) and accuracy was (74%). ROC curve of CoQ10 for pregnancy prediction is shown in Fig. 2.
Fig 2: ROC curve for serum Coenzyme Q10 for prediction of pregnancy (AUC=0.8).

Discussion

As we show in the current study, there was no significant difference in the mean dose of gonadotropin and duration of stimulation between pregnant or non-pregnant, which is in agreement with many other previous studies and these findings confirm the findings of other studies. (10-12)

However, it is not agreed with other studies that mentioned prolonged duration of gonadotropin stimulation is an independent negative predictor of ART success and obese women with BMI >30 kg/m2 required a higher dose of gonadotropin for stimulation and they face a lower likelihood of pregnancy after ICSI. (13,14)

As for oocytes characteristics in pregnant and non-pregnant ladies in the present study we found that there is a significant association were found between mean of number of abnormal and ruptured oocytes was in women who failed to get pregnant when compared to that of women who succeed to get pregnant. This is similar to the results were obtained by several other studies. (15, 16). But it is in contrast to another studies showed that the dark zona pellucida (DZP) does not affect the fertilization, embryo quality, or pregnancy rate. (17, 18)

In the present study, we found that there is significant difference in mean number of grade I and II embryos between pregnant and non-pregnant groups. Moreover, there was significant difference in mean number of grade III and IV embryos between pregnant and non-pregnant groups. which is similar to that found in may previous studies that have found better quality embryo and the number of embryos were statistically significant predictors of clinical pregnancy. (19,20)

The current study shows that the mean level of Coenzyme Q 10 in pregnant women was significantly increase than that in non-pregnant women. Turi A, et al, 2012 found that Protein levels of CoQ10/ concluded significantly in mature versus dysmorphic oocytes. Similarly, CoQ10/Cholesterol was significantly increase in grading I–II against grading III–IV embryos. (21) And total levels of CoQ10 were higher in follicular fluids related with mature oocyte and high-grade embryos, telling a possible correlation to the mechanisms of control and growth in follicular ambient. As reported in experimental in vitro cultures of myocardial cells, the CoQ10 stimulated the formation of ATP that in reproductive biology could accelerate formation of the blastocoeles cavity and consequently the hatching process (22, 23), second the existence of CoQ10 may precise ionic imbalance that happens in cultures of embryos. (24)

Gianmubilo SR et al, 2018 concluded that CoQ10 of oral supplementation might improve follicular fluid oxidative metabolism and oocyte quality, especially in over 35-year-old women. (25)

Conclusion

Significant decrease of Co Q10 between in Grade III and IV than that in Grade I and II, CoQ10 total were increased in pregnant women than non-pregnant

Conflicts of Interest: No

Source of Funding: Self

Ethical Clearance: was taken from the scientific committee of the Iraqi Ministry of health

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Effects of VEGF Gene Polymorphisms on BEV Responsiveness in a Sample of Iraqi Colorectal Cancers Using HRM - PCR

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Abstract

Background: Vascular endothelial growth factor (VEGF) have a vital role in the molecular genetic events of angiogenesis and vasculogenesis, so it is involved in the development of cancer. Single nucleotide polymorphisms (SNP) in VEGF gene has been announced as a risk factor in colorectal cancer. Bevacizumab (BEV) is an angiogenesis inhibitor that curb the binding of VEGF to its receptors obstructing the angiogenesis process. Objective: The ideal goal of ongoing study lies in revealing the effect of rs699947 (-2578 C/A) and rs833061 (-460C/T) polymorphisms in the promoter of VEGF gene on the development of colorectal cancer and on the BEV responsiveness in a sample of Iraqi patients using High Resolution Melting Analysis (HRM) analysis. Methodology: Venous blood samples were collected from 25 colorectal cancer patients with response to BEV treatment and 25 with BEV resistant and 25 apparently healthy individuals as control group who matched with patients in age and gender. Results: AA and CA polymorphisms A allele of rs699947 (-2578 C/A) and TT and CT polymorphisms and T allele of rs833061 (-460C/T) were represent a risk factor on the occurrence of the colorectal cancer. It has been found that CC and CA polymorphism of the VEGF - 2578 C/A and CT genotype of the VEGF -460 C/T polymorphism might be a predictive factors of responsiveness to BEV chemotherapy in CRC patients. Conclusion: These outcomes confirm the essential role that VEGF polymorphisms play in the occurrence of CRC and the correlation between SNPs in VEGF promoter region and the BEV responsiveness. With this, further research and investigation of VEGF polymorphisms could allow for its use in identifying risk factors for the development of CRC and increasing its predictive value for anti-VEGF cancer therapies.

Key word: VEGF Gene, Polymorphisms, BEV Therapeutic, Colorectal Cancers, HRM – PCR.

Introduction

Angiogenesis, the growth of new blood vessels from preexisting vessels, is an important process in physiological conditions, such as wound healing and tissue regeneration, or pathological conditions, such as heart disease and tumor formation. Vascular tumor generation (angiogenesis) is an essential process play an important role in the occurrence of tumor and metastases. It is controlled by the complex and coordinated procedures of pro-angiogenic factors and their receptors that become upregulated during tumor formation. Cancer cells are characterized by their ability to secrete many defective growth factors that contribute to stimulating the formation of neoplastic vessels. Among them, the VEGF represents one of the major powerful endothelial cell generators, and one of the most important triggers for vascular formation in CRC. Vascular endothelial growth factor (VEGF) is a signaling protein that contributes to the growth of new blood vessels. VEGF plays an important role in the process of blood flow to cells and tissues when there is hypoxemia caused by poor blood circulation. Thus, VEGF have an intrinsic role in both vasculogenesis and the formation of tumor blood vessels. On the other hand, inhibiting the process of formation of neoplastic hemangiomas contributes to reducing tumor formation.
One of the angiogenesis inhibitors is Bevacizumab (BEV), which represents a humanized antibody that is used to target the VEGF gene. For this treatment is used with many types of cancer, including colorectal cancer. Many single-nucleotide polymorphisms (SNPs) were demonstrated to regulate VEGF expression. Of that, rs833061 and rs699947 are two substantial SNPs located in the VEGF promoter gene, which might affect on promoter activity.

Methods

This study was carried out on a total of 75 subjects included 50 Iraqi patients with colorectal cancer who attended Alamal National Hospital and Oncology Teaching Hospital Baghdad during the period extended from the first of November /2019 to the end of January/2020, with age ranged from 25-86, and 25 apparently healthy voluntaries matched the patients group with age and gender.

Samples collection

Amount of three ml of venous blood was withdrawn from each subject under aseptic conditions after informing them about the aim of the research and filling in the questionnaire forms by them. Two ml of blood was placed in EDTA tube (1.5 mg/ml) and kept at -20 C° to be used in molecular study.

Genomic DNA was automatedly extracted from the whole blood samples of all subjects by using Blood DNA Extraction Kit 200 (MagPurix/Taiwan). The MagPurix technology is a state of the art platform that uses magnetic beads to extract nucleic acids from samples. The platform commits a truly walk-away automation in nucleic acid purification from samples to results. The purification process contains steps of lysis, binding, washing and elution. After genomic DNA was extracted, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA.

The qRT-PCR-HRM assay with specific primers (Alpha DNA / Montreal , Canada) designed with the Prime 3 software as shown in Table 1, was used to identify SNPs rs833061 (- 460 C / T) and rs699947 (- 2578 C / A) in the promoter region of the VEGF gene to investigate effects of VEGF polymorphism on CRC occurrence and the clinical response to BEV of CRC patients, using a specific primers and positive and negative controls that ensures a high degree of specificity. The program of HRM assay shown in table 2.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primers</th>
<th>Primer sequences 5’ - 3’</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs699947</td>
<td>F</td>
<td>5’ TTCCCATTCAGTCCAT 3’</td>
<td>88bp</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5’ CAGTCAGTCTGATTATCCA 3’</td>
<td></td>
</tr>
<tr>
<td>rs833061</td>
<td>F</td>
<td>5’ TCTGTGTGGGTGAGTGAG 3’</td>
<td>85bp</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5’ TATTGGAATCTGGAGTGAG 3’</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: HRM program

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95 °C</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95 °C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>Anneal</td>
<td>56 °C</td>
<td>60 seconds</td>
<td>35</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>Melting curve Analysis</td>
<td>72-95 °C</td>
<td>0.3°C/S</td>
<td>1</td>
</tr>
</tbody>
</table>

Result and Discussion

In order to estimate the effects of SNPs of VEGFA gene on response to BEV therapeutic in Iraqi colorectal cancer, results of rs699947 and rs833061 SNPs were compared. The findings of this study presented that AA and CA genotypes, and A allele of VEGF rs699947 were significantly increased the risk of colorectal cancer in patients group rather than control group (O.R. = 1.766; p = 0.0001). While, CC polymorphism and C allele was elevated in control group significantly (P≤0.01) when compared with patients group, considering a protective factor for them (table 3). This result is consistent with what has been reached by Jannuzzi et al. (2015), who found that −2578A>C was altogether connected with CRC chance.

When comparing the group of patients who are response to treatment of BEV with those who were resist to this treatment, it has been found that CC and CA polymorphism of the VEGF - 2578 C/A was highly significant associated with BEV responsiveness (O.R =1.307; p= 0.0006) and C allele frequency (0.52) was significantly higher in response group, pointing to the BEV response marker in CRC patients was CC and CA genotype and C allele (table 4). This is completely consistent with the research findings of Wang et al. (2015), as he showed that CC genotype has a close relationship of the therapeutic response to BEV treatment.

An opposite results was obtained in rs833061 SNP analysis, that TT and CT genotypes increase in patients group than in control group (O.R. = 1.397; P=0.0001) and T allele was significantly higher in patients group, indicating that TT and CT genotypes and T allele were a risk marker for the occurrence of CRC, while, CC genotype and C allele was significant higher in control group to be a protective factor for them (O.R.=1.754; P = 0.0001) as illustrated in table 5. Whereas, Jannuzzi et al. (2015) pointed that the distribution of -460 C> T between patients and controls did not differ significantly.

In other hand, CT genotype of the VEGF -460 C/T polymorphism might be predictive factors of responsiveness to BEV chemotherapy in CRC patients (1.703, p= 0.0001) as shown in table 6. In addition, C allele was significantly (P= 0.015) associated with BEV responsiveness. This is in line with the Wang et al. (2015) conclusion in identifying CT genotype as a therapeutic response factor to the BEV treatment.

Blood vessels are important for tumores to get its nutrition and oxygen to be developed and grow. A major mediator of angiogenesis in cancer is VEGF, it has the powerful action to boost the angiogenesis. BEV targeted VEGF, enervate or blocks the interaction between VEGF and its receptor, In doing so, it would be discourage endothelial cell proliferation, ending in knock down the angiogenesis in tumorigenesis. Finally, suppression of angiogenesis could prohibit the growth of...
tumors and enhance the therapeutic efficiency. 14

Since CRC is one of the most malignant cancers in the large intestine, it has the highest incidence and mortality. 15

The therapeutic efficacy of the response to BEV therapy is divers in patients who receiving the same BEV therapy. This event may be determined by the individual genetic variation. According to reports published, single nucleotide polymorphisms in VEGFA gene plays a crucial role in the development of tumors and cancer, of them CRC. The therapeutic competence of BEV may influenced by the SNPs in VEGF gene. 16

The most two vastly investigated SNPs in the promoter region of VEGFA gene are rs833061 (−460C>T) and rs699947 (−2578C>A). Examinations specify that these SNPs could influence the promoter activity of the VEGFA gene, and afterward change the VEGF expression.

**Conclusion**

As a goal of BEV treatment, abnormal VAGFA accumulate may lead to angiogenesis, leading to therapeutic disappointments. Although VEGF has been targeted by BEV, the polymorphisms at the promoter region of VAGFA gene may cause accumulation of its product and lead to angiogenesis and subsequently may fundamentally impact the helpful adequacy of BEV chemotherapy. 17,18

**Conflict of Interest:** None

**Funding:** Self

**Ethical Clearance:** Not required

| Table 3 : Distribution of genotypes and allele frequency of SNP rs699947 in difference groups |

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes</th>
<th>O.R.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
</tr>
<tr>
<td>Patients (No = 50)</td>
<td>12 (24.00%)</td>
<td>6 (12.00%)</td>
<td>32 (64.00%)</td>
</tr>
<tr>
<td>Control (No = 25)</td>
<td>17 (68.00%)</td>
<td>2 (8.00%)</td>
<td>6 (24.00%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001 **</td>
<td>0.348 NS</td>
<td>0.0001 **</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

** (P≤0.01)-HS.
Table 4: Relationship between rs699947 SNP genotypes and allele frequency with BEV responsiveness.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes</th>
<th>O.R.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
</tr>
<tr>
<td>Resistant patients (No = 25)</td>
<td>1 (4.00%)</td>
<td>2 (8.00%)</td>
<td>22 (88.00%)</td>
</tr>
<tr>
<td>Response patients (No = 25)</td>
<td>11 (44.00%)</td>
<td>4 (16.00%)</td>
<td>10 (40.00%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001 **</td>
<td>0.0473 *</td>
<td>0.0001 **</td>
</tr>
</tbody>
</table>

Allele frequency

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>0.08</td>
<td>0.92</td>
</tr>
<tr>
<td>Response</td>
<td>0.52</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* (P≤0.05)-S., ** (P≤0.01)-HS.

Table 5: Distribution of Genotype and allele frequency of SNP rs833061 in difference groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes</th>
<th>O.R.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>Patients (No = 50)</td>
<td>24 (48.00%)</td>
<td>24 (48.00%)</td>
<td>2 (4.00%)</td>
</tr>
<tr>
<td>Control (No = 25)</td>
<td>16 (64.00%)</td>
<td>9 (36.00%)</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0218 *</td>
<td>0.0457 *</td>
<td>0.308 NS</td>
</tr>
</tbody>
</table>

Allele frequency

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>0.72</td>
<td>0.28</td>
</tr>
<tr>
<td>Control</td>
<td>0.82</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* (P≤0.05)-S., ** (P≤0.01)-HS.
Table 6: Relationship between rs833061 SNP genotypes and allele frequency with BEV responsiveness.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes</th>
<th>O.R.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>Resistant patients (No = 25)</td>
<td>12 (48.00%)</td>
<td>11 (44.00%)</td>
<td>2 (8.00%)</td>
</tr>
<tr>
<td>Response patients (No = 25)</td>
<td>12 (48.00%)</td>
<td>13 (52.00%)</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>P-value</td>
<td>NS</td>
<td>0.0225 *</td>
<td>0.0489 *</td>
</tr>
</tbody>
</table>

Allele frequency

<table>
<thead>
<tr>
<th>Genotype</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant patients</td>
<td>0.70</td>
<td>0.30</td>
</tr>
<tr>
<td>Response patients</td>
<td>0.74</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* (P≤0.015)-S., ** (P≤0.01)-HS.

References


Development of Quick Reference Manual for the Management of Drug Overdose and Poisoning

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\textsuperscript{1}Department of Pharmacy Practice, J.K.K.Nattraja College of Pharmacy, Kumarapalayam, Namakkal, TamilNadu, India

Abstract

\textbf{Aim:} To develop a reference manual for the management of drug overdose and poisoning.

\textbf{Methods:} A prospective developmental study was performed for a span of six months at a tertiary care hospital in Erode. Information on the management of cases of drug overdose and poisoning was provided based on queries received at the Drug Information Center and the poisoning cases identified in the respective hospital over the past three years. Further attention was paid to detecting local poisons. Such information was collected on the basis of literature reviews, magazines and newspapers. In addition to drug and poison monographs; android applications, toxicological databases and links, standard reference books and articles on drug overdose and poisoning were included. Locally reported cases have been given more focus.

\textbf{Results:} A formulary was prepared which includes the management information for 100 drugs and 57 toxic substances that were identified after extensive research. The signs and symptoms of the various poisoning cases were graded as mild, moderate and severe based on poison severity scale (PSS) and respective antidotes were mentioned.

\textbf{Conclusion:} As per our knowledge, this is the first formulary that focus on local poisons. The implementation of the formulary may have a significant impact on healthcare professionals to improve the quality of life of patients. This provides important information of localized poisoning and drug overdose in a nutshell and therefore offers as a quick data to the enquirer.

\textbf{Key words:} Drug overdose, Formulary, Monograph, Poisoning

Introduction

Poisoning and drug overdose are important health problems in developing countries.\textsuperscript{1} The WHO reports annually that 0.3 million people are killed by specific poisoning agents. Acute pesticide poisoning is one of the most common causes of intentional death worldwide.\textsuperscript{2} High doses of analgesics, tranquilizers and antidepressants are widely used for intentional poisoning in industrialized countries and agricultural pesticides are used in Asia for self-poisoning, especially in rural areas with a fatality range of 10-20 percent.\textsuperscript{3,4}

Almost a million people die each year as a result of suicide, and chemicals account for a significant number of these deaths. An estimated 370,000 deaths each year are caused by deliberate ingestion of pesticides.\textsuperscript{5} On average, because of an accidental drug overdose, India loses at least two people every day. According to the latest data released by the Ministry of Health and Family Welfare, in three years from January 2014 to January 2016, 2381 people died from drug overdose, with 5 states responsible for 53% of all cases. Tamil Nadu peaks at 20%, followed by Punjab with 15%.

In India, especially in southern India, the incidence of drug overdose poisoning, organophosphates, and plant poisoning is growing. Tamil Nadu is topping deaths because of a drug overdose, according to the National Crime Records Bureau.\textsuperscript{6,7}
To save the patient’s life, providing timely information about poison and drug toxicity is very important. Poison information centers are a ready source of help in handling poisoning cases as they can provide reliable first aid information and guidance and emergencies related to chemical exposure. For developing nations, the number of poison and drug information centers is limited relative to developed nations. The number of poison centers that operate 24x7 for our country, particularly in southern India, is very minimal. In addition to the poison information centers, hospital formularies play an important role in the management of drug overdose and poisoning.

In developed nations, hospitals have own formularies that include local and regional poison management information. A hospital formulary on the treatment for drug overdose and poisoning is very limited in our region. In order to provide reliable and timely information, a quick reference manual is essential. Hence the objective of the study was to prepare a quick reference manual for drug overdose and poisoning treatment in the tertiary care hospital. As per our knowledge, this is the first poison management formulary in our region.

**Materials and Methods**

A prospective developmental study was performed for a span of six months at a tertiary care hospital in Erode. The endorsement of the study was obtained from the institutional ethics committee (JKNCP/ETHICS PRACTICE/019PDS07). Information regarding the management of drug overdose and management of poison cases were included based on queries received at the Drug Information and the poisoning cases reported at the respective hospital over the past three years. More attention has been paid to the detection of local poisons. Such information was collected on the basis of literature reviews, magazines and newspapers. Locally reported cases have been given more focus.

Monograph on all drugs and poisons in the formulary were prepared from primary, secondary and tertiary drug information resources. In addition to drug and poison monographs; android applications, toxicological databases and links, standard reference books and articles on drug overdose and poisoning were included. Depending on the ratings and reviews in the play store, android applications were selected.

**Results and Discussion**

Nearly 13 drug overdose and poisoning queries were collected from Drug Information Centre (DIC), out of which 2 were herbal related, 5 drugs and 2 insecticides queries as shown in Table 1. In addition, an extensive literature search was performed and categorized to local, South Indian and Indian poisons and it was found that in South India during a period of 2013-2018, there were 8 categories of herbal poisoning followed by 7 drugs and 4 insecticides queries. And in India during a period of 2013-2017, there were 25 categories of drug poisoning followed by 8 herbal, 6 others and 4 insecticides. Simultaneously, drug details of all the drugs that were available in the hospital were collected to suggest an antidote, in case of overdose. Along with this poison information scale or poison severity scale, a list of toxicological databases and links, lists of standard reference books regarding poisoning and toxicity monitoring, lists of android applications regarding poisoning and drug overdose, Journals regarding primary poison information and Poison Information Centers in India were included in the formulary.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Queries</th>
<th>Number of Queries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Herbal</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>Drugs</td>
<td>5</td>
</tr>
<tr>
<td>3.</td>
<td>Insecticides</td>
<td>2</td>
</tr>
<tr>
<td>4.</td>
<td>Others</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1: Number of Queries received in DIC Tertiary care Hospital Erode, regarding Poisonous substances (January 2015 – February 2018)
Drug Monographs contains the details of 100 drugs that could produce toxicity due to overdose and were classified based on their pharmacological class in which, 18 were in the category central nervous system, 16 in cardiovascular system, 14 in respiratory system, 13 in endocrine system, 12 in anti-infective, 9 in obstetrics, gynaecology and urinary tract disorder, 7 in musculoskeletal and joint disease, 6 in gastrointestinal system and 5 in anaesthetics as shown in Table 2.

Table 2: Pharmacological Class wise distribution of drugs in the formulary

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Drugs Category</th>
<th>Number of drugs (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gastrointestinal system</td>
<td>06</td>
</tr>
<tr>
<td>2.</td>
<td>Cardiovascular system</td>
<td>16</td>
</tr>
<tr>
<td>3.</td>
<td>Respiratory system</td>
<td>14</td>
</tr>
<tr>
<td>4.</td>
<td>Central nervous system</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td>Anti-infectives</td>
<td>12</td>
</tr>
<tr>
<td>6.</td>
<td>Endocrine system</td>
<td>13</td>
</tr>
<tr>
<td>7.</td>
<td>Obstetrics, Gynaecology and Urinary tract disorders</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Musculoskeletal and joint disease</td>
<td>07</td>
</tr>
<tr>
<td>9.</td>
<td>Anaesthetics</td>
<td>05</td>
</tr>
</tbody>
</table>

About 57 poisonous substances were included in the monograph and they are also classified, in which 12 were blood agents followed by 11 industrial chemicals, 10 herbals, 8 organ-phosphates, 5 carbamates, 4 organo-chorides and 2 environmental substances as shown from Table 3. Furthermore, if there is a chance of developing any toxicity, the treatment was included for the same.

Table 3: Class wise distribution of Poisonous substances in the formulary

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Poisonous substances</th>
<th>Number of substances (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blood agents</td>
<td>12</td>
</tr>
<tr>
<td>2.</td>
<td>Carbamates</td>
<td>5</td>
</tr>
<tr>
<td>3.</td>
<td>Herbal</td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td>Environmental substances</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>Industrial Chemicals</td>
<td>11</td>
</tr>
<tr>
<td>6.</td>
<td>Organophosphates</td>
<td>8</td>
</tr>
<tr>
<td>7.</td>
<td>Organochlorides</td>
<td>4</td>
</tr>
<tr>
<td>8.</td>
<td>Other Chemicals</td>
<td>5</td>
</tr>
</tbody>
</table>
Monograph content: While considering the Drug section, the particular drug comprises the following details: Name, Functional Class, Mechanism of Action, Uses, Unlabeled Uses, Contraindications, Precautions, Dosage and Routes, Adverse Effects, Signs and Symptoms of Toxicity and Treatment overview. The Monograph of the particular poison contains the following details: Name, Synonym, Description, Clinical effects, Treatment overview. Signs and symptoms of toxicity are classified into mild, moderate and severe by using Poison Severity Scale (PSS).

Poison Information Scale or Poison severity scale: A standardized poison rating scale allows for qualitative assessment of morbidity caused by poisoning, better detection of real risks and comparability of results. The PSS has been published externally. PSS helps in the initial diagnosis and assessment of drug overdose and poisoning.

Toxicological Databases and Links: For further references, toxicological databases and their respective links are provided for easy access to detailed information.

Journals Regarding Poison Information: 19 international poison information standard journals issued by different medical associations are included.

Standard Reference books regarding poisoning and toxicity monitoring: Details of Standard reference books on Toxicology, Pediatric toxicology diagnosis and management, Poisoning and drug overdose, Pharmacopoeias, Formularies, Manuals of toxicologic emergencies are included.

Android Applications: Easily access able android applications detailing poisoning and drug overdose are included in the formulary. These applications were selected based on the user’s reviews and ratings. These applications are innovative, online triage tools and guide users faced with poison emergency through a series of simple questions to denote the toxicity provides a quick guide on poisoning and antidotes.

Table 4: List of Android applications regarding poisoning and toxicity monitoring included in the formulary

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Mobile Applications</th>
<th>Offered by</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>webPOISONCONTROL</td>
<td>National Capital Poison Centre</td>
</tr>
<tr>
<td>2.</td>
<td>Poison Rx</td>
<td>PharmITexpert</td>
</tr>
<tr>
<td>3.</td>
<td>Poisoning and drug Overdose</td>
<td>MobiSystems</td>
</tr>
<tr>
<td>4.</td>
<td>Poisoning &amp; Drug Overdose ref.</td>
<td>AgileMD</td>
</tr>
<tr>
<td>5.</td>
<td>Poison information Centre</td>
<td>JSS Academy of higher Education and Research</td>
</tr>
<tr>
<td>6.</td>
<td>Poison First Aid for Children</td>
<td>Kigorosa UG</td>
</tr>
</tbody>
</table>

Poisoning is reported to be a major global public health issue. A developed country may have its own information services for its unique needs, but the Poison Information Center where it exists may be the only source of information accessible 24 hours a day on toxic chemicals in a developing country. The reality that there are fewer poison knowledge centers in India is appalling to notice. Due to the lack of information on poisoning cases in India, the introduction of new guidelines and the updating of existing protocols requires knowledge.
of demographics and the management of poisoning cases. It is necessary to understand the interventions needed in the management of poisoning and to define the role of health professional studies. Knowledge of the general pattern of poisoning in a particular region can help reduce mortality and morbidity rates in the early diagnosis and treatment of cases.

Conclusion

Drug overdose and poisoning is a serious problem that arises whether deliberately or accidentally. An overdose can be mild, moderate, or extreme. Symptoms, treatment, and recovery depend on the specific drug involved. Medical professionals are implementing new approaches for diagnosis and treatment due to the alarming rate of a drug overdose and poisoning, especially in developing countries. The introduction of new guidelines and the updating of existing protocols requires knowledge of demographics and the management of poisoning cases due to the lack of information on poisoning cases in India. The strategies needed to manage to poison need to be identified and the role of health professional studies established. This formulary provides the important details of localized poisoning and drug overdose into a nutshell which is easy to access and thereby provides the enquirer with quick information. The formulary is handyuser-friendly, and saves the precious time of busy physicians. It may also promote the safe and effective use of medicines thereby minimizing drug-related problems in the population.

Ethical Clearance: Ethical clearance and approval was obtained from J.K.K.Nattraja Institutional Ethical committee with reference number JKKNCP/ETHICS_Practice/019PDS07.

Conflict of Interests: The authors declare no conflict of interest.

Source of Funding: Self

References

Evaluation of immunoglobulins IgG, IgM, IgA and Complement Components C3 and C4 Levels in Iraqi full term Neonates with Severe Hyperbilirubinemia

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1Department of Chemistry and Biochemistry, College of Medicine, Mustansiriyah University, Iraq,
2Paediatrician in Central Children Teaching Hospital

Abstract

Background and aim: Hyperbilirubinemia is an elevation of the bilirubin levels in blood of newborn babies that presented as jaundice. This study aimed to evaluate the levels of important of immunological system proteins (IgG, IgM, IgA, C3 and C4) in both newborn babies with severe hyperbilirubinemia and healthy controls.

Methods: Thirty (30) full-term neonates with severe hyperbilirubinemia (serum bilirubin >20 mg/dl) and twenty five (25) healthy control full-term neonates were included in the study. Blood samples taken and Hb, total serum bilirubin, total protein measured immediately by spectrophotometer. IgG, IgM, IgA, C3 and C4 measured by immune-nephelometric method.

Results: In hyperbilirubinemia group (Male/ Female percentages) were (63% / 37%), (IgG 373.4±218 mg/dl), (IgM 20.5 ± 24.03 mg/dl), and (C3 209.4±17.81 mg/dl) values were significantly lower than control group ( 588.2±298.5 mg/dl IgG, 26.44 ± 2.92 mg/dl IgM and 627.5±221.1 mg/dl for C3). Correlation analysis revealed that in control group IgG, IgM, and C3 shown to be significantly (P value <0.05) and positively correlated (r-value >3) with both age and weight.

Conclusions: jaundice is a risk factor for sepsis that could be due to lower levels of immunoglobulins and complements components. The normal development of immune system that observed with increasing age and weight was impaired in patients with neonatal hyperbilirubinemia.

Key words: Neonatal hyperbilirubinemia, Complement components, Immunoglobulins and Total serum bilirubin.

Introduction

Hyperbilirubinemia is one of the commonest clinical conditions in newborn babies that presented as jaundice (1). It affects both full-term and premature neonates in about 60% and 80% respectively (2, 3). However, most of the cases are physiological jaundice that is a safe and do not involve any clinical complications, but excessive elevation of unconjugated bilirubin has a potential development of neurotoxicity and kernicterus. A variable risk factors have been proposed for neonatal hyperbilirubinemia that includes; genetic, maternal, prenatal, neonatal and other factors (4).

Physiologically, bilirubin is produced and elevated in neonatal life as a results of excessive distraction of fetal red blood cells (5). These resultant molecules are not merely bi products of a heam metabolism rather than its potential cytoprotective role and has an antioxidant activity such as that observed of serum uric acid (6, 7). Newborn baby is continuously produced free radicals even in the absence of any pathology , during this period the body is at great vulnerability of oxidative stress (8). Indeed, at this period different body antioxidant mechanisms are not fully matured and thus bilirubin at
physiological level is suggested as a defense mechanism against the harmfully generated reactive oxygen species (9). The other side it showed that exceeding limited level of bilirubin in neonatal period has an adverse effect on baby defense mechanisms. Studies had shown that a strong associations were observed between hyperbilirubinemia and risk of infection and septicemia (10, 11). Furthermore, accumulative evidences have demonstrated that, severe hyperbilirubinemia was associated with modulation of different components of immune response. Haga, Tempero (12) showed that intracellular bilirubin precipitation causes an inhibitory effect on T helper-1 cell and their inflammatory response. Neonates with hyperbilirubinemia showed an impairment of immunological response (decrease immunoglobulins production) to routine vaccination series against measles, diphtheria and tetanus (13). Neonates with hyperbilirubinemia showed a reduced Lymphocytes proliferation (14).

Both adaptive and innate immune responses are still immature during neonatal period (15). B lymphocytes, the origin of immunoglobulins, produced in late 1st trimester in fetal life but remain unable to produce antibodies or immunoglobulin class switching during early neonatal periods (16). Indeed, active IgG production starts around the third month of life but during neonatal period serum IgG represents maternal IgG transported via placenta to fetal blood. IgA in neonatal serum supplied via early breast feeding (17). Furthermore, studies observed that the components of complement system are under-developed in newborns as well (18).

Hyperbilirubinemia causes reduction of antibodies production, total, IgM and IgA levels (14). In addition, a strong inverse association was observed between elevated serum bilirubin and the development and maturation of complement cascade (19).

This study was set to evaluate the influence of severe hyperbilirubinemia in neonates, regardless its cause, on immunological markers represented by IgG, IgA, IgM and complement components C3 and C4.

**Patients and Methods**

The current cross-sectional study approved and conducted in Chemistry and Biochemistry Department/College of Medicine / Mustansiriyah University in 2019-2020. Thirty(30) full-term neonates were selected as cases with severe hyperbilirubinemia (serum bilirubin >20 mg/dl) in neonatal word during their preparation for phototherapy or exchange transfusion in the Central Child Teaching Hospital in Baghdad over the period from April - November 2019. Twenty-five (25) healthy control full-term neonates been chosen from an outpatient clinic of the same hospital for any presentation and shown not have jaundice as confirmed by total serum bilirubin (TSB) measures. For both groups, age, sex, gestational age and mother age recorded and body weight measured. Five (5 ml) of venous blood were with drowning of each individual and allowed to clot, or with drowning in heparin tubes or EDTA tubes. Hb, total serum bilirubin (TSB) and total serum protein measured immediately. Serum and plasma were stored at -20°C or tested immediately after collection and separation for total immunoglobulin (IgG, IgM, IgA, C3 and C4) measurements.

IgG, IgM, IgA and complement components C3 and C4 measurements:

Immunoglobulins and complement C3 and C4 measurements performed using nephelometric method; the device used for the assay was Beckman IMMAGE® Immunochemistry Systems using provided commercially available kits by Beckman Coulte, USA. The test performed according to the manufacturer’s protocol under controlled laboratory conditions. Note; no interference was observed between serum bilirubin and parameters measured.

Statistical Analysis

Analysis of data carried out using the available statistical package of Graph Pad Prism (California) software. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test ($\chi^2$-test). Statistical significance was considered whenever the P value was equal or less than 0.05.
Results

Hyperbilirubinemia affects male (63%) baby more than female (37%). The mean age jaundiced baby was (4.07±1.31) days and their TSB was (23.63±2.30 mg/dl) while (0.9± 0.24 md/dl) in control group, as shown in (Table 1). The mean levels of IgG in control group (588.2±298.5 mg/dl) was significantly higher (P value <0.01) than that found in hyperbilirubinemic babies (373.4±218 mg/dl), the rest of immunoglobulins and complement proteins results were showed in (Table 2) and (Figure 1). IgG, IgM, and C3 shown to be significantly (P value <0.05) and positively correlated (r-value > 3) with both age and weight of healthy control neonates as shown in (Figures, 2 and 3) where graphs representative of correlation analysis of neonatal age and weight with IgG, IgM and C3 in both controls and hyperbilirubinemic group.

Table 1: Characteristics of neonates with hyperbilirubinemia and controls.

<table>
<thead>
<tr>
<th></th>
<th>Severe Hyperbilirubinemia</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>4.07±1.31 (2-7)</td>
<td>3.84 ±1.6 (2-7)</td>
<td>Ns*</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19 (63%)</td>
<td>16 (64%)</td>
<td>Ns*</td>
</tr>
<tr>
<td>Female</td>
<td>11 (37%)</td>
<td>9 (36%)</td>
<td>Ns*</td>
</tr>
<tr>
<td>Weight (gm)</td>
<td>2675±578.5 (1700-4000)</td>
<td>3383±526.9 (2240-4180)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Gestational age</td>
<td>37.97±1.54 (36-40)</td>
<td>38.76±1.56 (36-40)</td>
<td>Ns*</td>
</tr>
<tr>
<td>Mother age (years)</td>
<td>27.17±5.16 (19-37)</td>
<td>27.32±5.35 (19-37)</td>
<td>Ns*</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>17.33±1.47 (14-20)</td>
<td>16.88±1.59 (13.4-19.7)</td>
<td>Ns*</td>
</tr>
<tr>
<td>TSB (mg/dl)</td>
<td>23.63±2.30 (20-29)</td>
<td>0.9±0.24 (0.6-1.32)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total serum protein (gm/dl)</td>
<td>5.32±0.79 (4.1-6.8)</td>
<td>6.21±0.73 (5.01-7.48)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Significant difference between two independent means using Students-t-test at 0.05 level.
Table 2. Immunoglobulins and complement C3 and C4 for study subjects

<table>
<thead>
<tr>
<th></th>
<th>Severe Hyperbilirubinemia</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. No.</td>
<td>No. No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>Mean±SD (Range)</td>
<td>373.4±218 (81-827)</td>
<td>588.2±298.5 (167-1308)</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td></td>
<td>20.5 ± 24.03 (6-104)</td>
<td>26.44 ± 2.92 (9-121)</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td></td>
<td>10.1±5.9 (3-28)</td>
<td>6.5±5.5 (1-21)</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>209.4±17.81 (168-236)</td>
<td>627.5±221.1 (216-1118)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>82.1±25.24 (46-140)</td>
<td>186±71 (70-396)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Significant difference between two independent means using Students-t-test at 0.05 level.

Figure 1: Immunoglobulins and complement C3 and C4 for study subjects.
Figure 2: Graph representative of correlation analysis of neonatal age in days with IgG, IgM and C3 in both control and hyperbilirubinemic groups.
Discussion

This study aimed to compare the levels of immunoglobulins and complement components C3 and C4 in neonates with severe jaundice, regardless its cause, with aged matched healthy controls.

The main findings of this study were predicted that; in hyperbilirubinemia group, there were lower body weight and lower total protein level when compared to controls. In addition, low levels of IgG, C3 and C4, while high IgA levels were found in jaundiced neonates compared to controls. No significant differences were demonstrated of level of IgM between the two groups. Furthermore, the strong positive correlation that observed in control group between body weight and age with each of IgG, IgM and C3 was disturbed in babies with hyperbilirubinemia.

Hyperbilirubinemia in newborn babies is usually due to elevation of unconjugated (indirect) bilirubin that accrued as results of increased bilirubin production over conjugation and hepatic clearance of bilirubin (20). The clinical classification of significant hyperbilirubinemia could categorize according to bilirubin level as;
Significant hyperbilirubinemia (TSB ≥12) mg/dL, Severe hyperbilirubinemia (TSB ≥20) mg/dL. Extreme hyperbilirubinemia typically (TSB ≥25) mg/dL and hazardous or critical hyperbilirubinemia (TSB ≥30) mg/dL (1). In the current study the hyperbilirubinaemic neonates group was either severe or extreme class, TSB (23.63±2.30) mg/dl, the range of TSB readings was (20-29) mg/dl, those admitted to neonatal word for either phototherapy or exchange transfusion treatment.

Results of the current study showed that, neonatal hyperbilirubinemia affects male more than female, more than 60% of hyperbilirubin group were male and they tend to have higher serum bilirubin level (24.1±2.4) mg/dl than female (22.8±2.0) mg/dl however this was statistically not significant. These results come in agreements with many studies including, Al-Banna, Riad (21) and Greco, Arnolda (22). The exact causes for this gender differences are unknown but different mechanisms been suggested. Tioseco, Aly (23) attributes the sex difference due to that dysfunction of placenta, which is more common during pregnancy in male fetus which could contribute to higher risk of early life jaundice. Furthermore, during fetal life, male fetus has higher metabolic rate than female which could contribute to the elevated turnover of bilirubin in male newborn baby (24).

Both Immunoglobulins and complements components are proteins that were detected in the plasma and body fluids and the play a significant role in protection from infections. and the knowledge of these components of immune system in neonates is vital in detection of immunological as well as infectious disease (25).

In control group, the level of IgG and IgM were comparable with study conducted by Kardar, Oraei (26). They found that mean IgG was (507.6 mg/dl, IgM 26 mg/dl, IgA 5.6 mg/dl, C3 690 and C4 was 167 mg/dl), which was so close to present results. The current study values were (588.2±298.5 mg/dl for IgG, 26.44 ± 2.92 mg/dl for IgM, IgA was 6.5±5.5 mg/dl, C3 627.5±221.1 mg/dl and C4 186±71 mg/dl). Another important study conducted in Turkey published in 2015 by Alkan Ozdemir, Ozer (27) also confirmed what this study found. Their finding regarding IgG, IgM and their correlation with body weight and gestational age. IgG was 791.5±234.9 mg/dl and IgM 10.6±6.7 mg/dl that seem to be slightly higher to the findings of our study. Strong positive correlation observed between body weight and age with these parameters. The correlation measures of the current study was similar and found that IgG, IgM and C3 protein level were strongly and positively correlated with age and weight with P value <0.05.

To our knowledge, this is the first study conducted in Iraq comparing immunoglobulins and complement factors in hyperbilirubinaemic neonates. No recent published data for the last ten years world widely covered this subject even thorough exploration to the scientific database.

Low level of serum Bilirubin in early fetal life has a strong beneficial effect and it acts as antioxidant, but evolving evidences have observed that at severe hyperbilirubinemia could act as risk factors for disease and injury (28, 29). Furthermore, Old observation documented in Větvička, Šíma (30) and Jangi, Otterbein (14) showed that low levels of immunoglobulins and complement components were observed in neonates and adults with hyperbilirubinemia and affects their immunological status. The results of present study come in accordance with these findings. An attractive finding of the current study, the positive correlation that observed between age and weight of normal neonates with immune protein factors been lost in high bilirubin level group.

In conclusion, neonatal hyperbilirubinemia associated with an increasing risk of infection and injury that could be due to lower levels of immunoglobulins and complements components in affected neonates. Additionally, the elevation of immune related proteins with age and body weight of jaundiced baby was disturbed.

**Ethical Clearance:** Scientific committee of the Department of Chemistry and Biochemistry, under the rule of College of Medicine, Mustansiriyah University, ethically approves this work.

**Conflicts of Interest:** Researches in present work have no potential conflicts of interest relevant to this article.

**Source of Support:** This work is totally funded by...
the participated authors.

Acknowledgments: Deep thanks to the teaching staff of the Department of Chemistry and Biochemistry, College of Medicine, Mustansiriyah University for their scientific supports.

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Aetiology and Findings of Recurrent Chronic Rhino Sinusitis with Nasal Polyposis after Functional Endoscopic Sinus Surgery

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Abstract

Background: Nasal polyps are the edematous sinonasal mucosa prolapsing into the nasal cavity. Allergy, asthma, aspirin intolerance and fungal rhinosinusitis have been implicated in various studies. Recurrence of nasal polyposis after functional endoscopic sinus surgery is prevalent but there are no specific causes for recurrence. Intensity of relapse varies depending on multiple factors that implicated in showing characters and findings of the recurrence.

Objective: To evaluate causes of nasal polyposis recurrence after FESS.

Patients and method: 30 adult patients age between 18 and 60 years (with recurrent nasal polyposis after FESS) were studied in the period between March 2017 and September 2018. Assessment done for all patients by taking Medical history, documented data from the primary surgery, rhinoscopy with CT-scan examination.

Results: In this study, most of nasal polyp recurrence occur in 1-3 years after primary surgery, patients using post-operative steroid showed delay in recurrence, post-operative antibiotic showed no effect on time of recurrence, patients with (asthma, allergy, aspirin sensitivity and allergic fungal rhinosinusitis) showed high Lund-kennedy and Lund-mackay scores and some patients had anatomical findings mainly (40% incomplete anterior ethoidectomy).

Conclusions: Recurrence of nasal polyposis could occur at any age mainly in middle age group and male gender showed higher prevalence. Asthma, aspirin sensitivity (Samter’s tried) and allergic fungal sinusitis were the most important associated diseases. CT-scan showed some anatomical variations like incomplete anterior and posterior ethmoidectomy which occupying the highest percentages. Post-operative steroid showed delay in time of recurrence.

Keywords: nasal polyp recurrence, functional endoscopic sinus surgery, nasal polyp associated with asthma, aspirin sensitivity and allergic fungal rhino sinusitis.

Introduction

Nasal polyps are edematous grapelike protrusions most often originating in the upper part of the nose around the osteomeatal complex on the lateral wall. The surface epithelium tends to be smooth and consists of pale translucent tissue which distinguishes them from the more vascular mucosa of the nasal cavity.

Polyps can vary widely in size and should be considered a bilateral condition. Rare cases of unilateral polyps should only be diagnosed once all other more likely pathologies have been reliably excluded (1)

Surgical management is considered for patients who have failed to respond to maximal medical treatment and for those with complications. Functional endoscopic sinus surgery (FESS) aims to improve sinus ventilation
and drainage as well as removing polyps

Embryology of the nose and PNS

Developmentally nose and paranasal sinuses are interlinked. They are always considered together developmentally. The various sinuses may follow different calendars but their origin is the same.

Nose and paranasal sinuses always considered together developmentally

Relevant anatomy

Nasal endoscopy of patients with nasal polyposis and anatomic examination of specimens have shown that nasal polyps are situated in the middle meatus and that they originate from the mucousa of paranasal sinuses outlets. This area, so critical for sinus pathology, and referred to as the ostiomeatal complex.

Nasal polyposis

Nasal polyps are the oedematous sino-nasal mucosa protruding into the nasal cavity. Simple nasal polyps are part of the spectrum of chronic rhinosinusitis and are formed by the sino-nasal lining becoming progressively more inflamed and thicker and then pedunculating into the nasal cavity.

Site of polyp formation:

Polyps usually arise from the lateral nasal wall in the middle meatus or sphenoid recess. Those polyps that originate lateral to the middle turbinate usually take their origin from the frontal, anterior ethmoid, or maxillary sinuses. Polyps medial to the middle turbinate are usually arising from the posterior ethmoid or sphenoid sinuses. In rare cases, polyps may originate on the nasal septum or from the olfactory cleft.

Diagnosis and staging

Rhinoscopy

Large polyps can be identified by simple anterior rhinoscopy. In contrast to a hyperplastic turbinate, a polyp can be made to move by touching with a probe, mainly senseless and not bleed in touch. Endoscopic examination with a rigid scope is the preferred examination, as it can diagnose even small polyps in the middle meatus and give a good assessment of the extent of the disease and of anatomical abnormalities.

Endoscopy is useful, not only for the diagnosis, but also for follow-up examination after medical and surgical treatment, and for staging of the disease.

Imaging

CT scan of the nose and paranasal sinuses gives a demonstration of the anatomy and even pathology. It is indicated when there is a suspicion of malignancy or meningocele, and also in all cases before endoscopic surgery. In addition, it is used for staging of the disease.

TREATMENT

Intranasal and systemic steroids are the most common treatments for the management of nasal polyps. Endoscopic surgery is reserved for severe nasal obstruction resistant to maximal medical therapy. Nasal polyps tend to be more severe and refractory to medical and surgical treatment, especially in the subset of aspirin-sensitive asthmatics.

Endoscopic polypectomy

For large nasal polyps or patients that fail medical treatment, endoscopic polypectomy with the microdebrider is performed.

Nasal polyposis recurrence

The recurrence of NP after the sinonasal surgery is prevalent but the frequency and intensity of relapse vary based on multiple factors: Medical factors, environmental factor like smoking and iatrogenic factors.
Fig. (1): (A) coronal CT scan of patient with Samter’s triad showing recurrence of polyps after FESS (7) (B) Sagittal CT scan of polyps recurrence (7)

Patients and Methods

All 30 patients in this study had previously undergone bilateral primary FESS procedure for CRSwNP. Independent variables which were assessed included patients name, age, gender, history of nasal obstruction, purulent nasal discharge, post nasal drip, facial pain, anosmia, headache, History of asthma and aspirin sensitivity and allergic fungal sinusitis.

Patients information were collected from outpatient, inpatient notes and primary FESS reports.

Nasal endoscopy using 0,70 degree Hopkins rod nasal endoscope for assessment of the nasal polyp by using Lund and Kennedy score for presence of polyps, edema, scarring, crusting and discharge.

Each side was graded separately, and the scores for each side were collected to determine the overall endoscopy score.

Fig. (2): Endoscopic examination of Rt. Nasal cavity by 0° endoscope.

Prior to revision surgery, prescription of maximal medical therapy for each patient included:

Ø Systemic oral steroid (0.5 mg/kg/d) for 5-10 days in case of massive polyposis.

Ø Local corticosteroid (budesonide) spray.

Ø Antihistamines for patients with history of allergy.
Ø Macrolide antibiotics (azithromycin tab. 500 mg. once daily for 3 days).

Ø Saline irrigations.

Medical treatment was given for 3-6 weeks before undergoing a preoperative CT scan and another endoscopy were done.

Revision ESS considered after one month of medical treatment failed.

All patients had preoperative computed tomography (CT) scans of the nose and paranasal sinuses (coronal, axial, and sagittal) and scoring done according to Lund McKay scoring system.

![CT-scan coronal sections of patient with recurrent nasal polyps after FESS](image)

**Fig. (3): CT-scan coronal sections of patient with recurrent nasal polyps after FESS**

**Table (1): Anatomical findings sheet:**

<table>
<thead>
<tr>
<th>Anatomic findings</th>
<th>Rt side (0-1)</th>
<th>Lt side (0-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>septal deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>middle turbinate laterization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>residual uncinate process</td>
<td></td>
<td></td>
</tr>
<tr>
<td>residual Haller (infraorbital) cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onodi (sphenoid) cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>residual cells in the frontal recess</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(agger nasi or frontoethmoidal cells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMA stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete anterior ethmoidectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete posterior ethmoidectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sphenoid sinus and its ostium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>concha bullosa (missed or incompletely opened)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each finding the incidence is identified in all patients included in the study.
Surgical procedure:

In reverse trendelenberg position, head elevation up to 30 degree, bilateral otrivin packs was inserted for about 10 minutes as to decrease congestion.

We used 0 degree nasal rigid endoscopes and endoscopic nasal suction tip, to remove polyps from the nasal cavity by using microdebrider and Blakesely weil cupped forceps, multiple pieces taken as biopsy.

After identification of remaining landmarks, we started removal of polyp and access all involved sinuses and remove the polyp as can as possible.

Correction of any anatomical abnormality if present like (septal deviation, middle turbinate lateralization, residual uncinate process, and other anatomical findings. then irrigation of the nasal cavity with normal saline and suction.

This follow up was done by examination of the nasal cavity endoscopically using 0, 70 degree angles, bilateral three passes nasal endoscopic examination done to identify edema, discharge, synechiae, crust and recurrence of nasal polyposis.

Result

Gender:

male to female ratio was 1.5:1.

Symptoms (chief complaint)

There were no dominant symptoms.

Asthma, aspirin sensitivity and AFRS

some patients associated with asthma, aspirin sensitivity and allergic fungal rhinosinusitis as shown in Fig.(21)

![Graph](image)

Fig.(4) patients numbers according to associated diseases.(asthma, aspirin sensitivity and AFRS).
Date of primary surgery

Our patients did the primary surgery in different times. The oldest one in 2010 and the newest one in 2016 so we divided the patients into 3 groups according to the year of primary surgery.

We found that the largest number of the patients in our study did the primary surgery in the years between 2014 and 2016, that means NP recurrence occurs mainly (1-3 years) after surgery.

Effect of post-operative steroid

We found that patients those received steroid (local or systemic) after primary surgery showed delay in polyp recurrence.

Anatomical findings

In some patients we found anatomical variations in CT scan and intra-operatively.

Incomplete anterior and post. ethmoidectomy had the largest percentage.

Discussion

Gender and age distribution

In our results male to female ratio was 1.5:1 just like what found by Manpreet Singh Nanda et al. when most patients were within the middle age group and M: F ratio of 1.6:1 (8) but not goes with Shabbir Akhtar et al. that showed the recurrences in 36 patients from 192 patients, and no association between polyp recurrence age, gender (9)

Symptoms:

In our result there were no dominant symptoms just like Studies done by Shabbir Akhtar et al. showed recurrences developed in 36 patients. No association of recurrence with sinonasal symptoms (9)

Also Adam S. DeConde et al. studied the factors may related to nasal polyp recurrence and addressed
nasal symptom among these factors and found that there was no clinical risk factors for that (10).

Asthma, aspirin sensitivity with ct-scan and endoscopic finding

Wynn et al. in study patients with severe polyposis, they found (50%) patients had asthma (11) and this goes with our results.

In the study done by Fereshteh Esmatinia and Mahdi Bakhshaei, 80% of patients with asthma who had been underwent the ESS revealed the Sino-nasal polyposis recurrence (12) And this goes with but higher than our results.

AFRS: Study done by Younis RT, Ahmed J. found that patients with polyps in AFRS had high Lund-Mackay score and had higher revision rates. This does with our result (13)

Study done by Laila M. Telmesani showed there was a significant direct relationship between the CT grading of nasal polyps and polyp recurrence in nasal polyposis with and without AFRS (14) this goes with our results.

Figen Aslan et al. found the mean Lund–MacKay CT score and the mean Lund–Kennedy endoscopic score were significantly higher in patients with AFRS (15) And this consistent with our results

Date of primary surgery and recurrence

We found that the largest number of the patients in our study did the primary surgery 1-3 years after primary surgery. (The most recent period).

In study done by DeConde AS et al. Polyp recurrence is common after ESS, and the high recurrence rate (60%-70%) occurred just post-operative up to 18 month (10) and this goes with our result. In Yaniv Eitan et al. from 148 patients underwent FESS, polyp recurrence occurred in 74 patients in the first 2 years after surgery and an additional 17 in the next 3-4 years. During the following years, the recurrence rate was lower (16) and this also goes with our result.

Pär Stjärne et al. found the recurrence occur mainly about one year after primary surgery and this result consistent with our result (17)

Effect of post-operative steroid on polyp recurrence rate

Esmatinia et al. only 46% of patients consumed beclomethasone dipropionate, revealed the NP regrowth which was lower than the 87% of patients in placebo group that revealed recurrence of NP (12)

Fandiño C. et al. they found using of intranasal corticosteroid showed significant improvement in polyp score (18)

Anatomical findings

During examination of our patient by endoscopy and using of CT scan and intraoperative we found that some patients had anatomical variations contribute in failure of primary surgery and we documented that as an incidence in bilateral assessed sides and found:Incomplete anterior ethmoidectomy in 40%, Incomplete posterior ethmoidectomy in 36%, MMA stenosis in 30 %, Middle turbinate lateralization in 22%, Residual uncinate process in 20%, Septal deviation in 17%.

Khalil et al. They found residual anterior and posterior ethmoid cells in 97% and 92% of patients, respectively (96% and 92% of sides, respectively) (19)

Ramadan et al. reported a series of 52 patients prepared for revision FESS, among these, he observed (residual uncinate) in 15% (20)

Adam S. DeConde et al. mentioned that the examination of the association between surgical extent and polyp recurrence in a previous single institution cohort study found that more extensive surgery was protective against polyp recurrence (10) and this goes with our result.

Conclusion

ü Most of nasal polyposis recurrence occurs in (1-3 years) after FESS.

ü Asthma, aspirin sensitivity (Samter’s tried) and allergic fungal sinusitis were the most important associated diseases.

ü CT-scan of patient with recurrent nasal polyposis showed some anatomical variations from the primary surgery, incomplete anterior and posterior
ethmoidectomy showed the highest percentages.

ü According to the date of primary surgery, patients who received post-operative steroid showed delay in time of recurrence unlike other patients who did not receive steroid so recurrence appeared earlier.

ü Medical treatment post-operative is mandatory by nasal wash, local steroid and close follow up to decrease the rate of recurrence.

**Recommendations**

ü Meticulous dissection during surgery and wide surgical exposure of nasal sinuses and removal of polypoidal mucosa are the most important points for successful surgical removal and decrease of polyp recurrence.

ü Post-operative regular and frequent follow-up is important to assess operative field for any infection, edema, crust and polyp recurrence.

ü We recommend use of post-operative steroid which showed to be so important to decrease the chance of nasal polyposis recurrence or even delay the recurrence.

ü Further study of large number of patients and long time is needed to show other etiological factors and possible ways to prevent or decrease polyp recurrence.

**Source of Founding:** Self-source

**Ethical Clearance:** Taken From Iraqi board for medical specializations in otolaryngology.

**Conflict of Interest:** Nil

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Review: Cancer Cells Resistance Strategies

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Abstract

Cancer, which is the most important health problem, develops the ability to resistant traditional medications this leads to increase efforts to develop new cancer medication protocols. In this review, the chemotherapy resistance could be due to several mechanisms. These mechanisms involve either inactivation by two important phases, phase I which involve oxidation, reduction and hydrolysis or phase II which involve conjugation wi, alteration of the target site which decrease either activation of anticancer prodrug and/or decrease its influx to cancer cells. Other mechanisms for chemotherapy resistance return to the repairing of cancer cell DNA, modulation of efflux for drug, inactivation of apoptosis, besides how the role of heterogeneity of tumor cells in drug resistance. All of these strategies obligated the oncologist to use a combination of chemotherapy medication to overcome or avoid resistances to treatments.

Keywords: cancer, resistance, inactivation, apoptosis, DNA damage

Introduction

Cancer research provides important information about its biological characteristics, this information is updated every day. As a disease, cancer is characterized by many features such as the uncontrolled proliferation of abnormal cells and dynamic changes in the genetic materials (¹). The progression of malignant cells overwhelms normal cell divisions. This overwhelming is achieved by the invasion of normal tissues nearby and metastasize to distant tissues (²). Conventional cancer treatments such as surgery for removing cancer mass, ionic radiation therapy, chemotherapy therapy, combination therapy, and laser therapy show improvement for several cases but with high tendencies for side effects, the selective therapies which depend on the other origin like genetically targeting in the tumor progression used for the promising treatments with fewer side effects (³). Chemotherapy has been using for decays with an acceptable degree of success, this success sometimes flips into a failure due to cancer cell resistance, 90% of chemotherapy treatment in advance cases of cancer disease have been failed due to this resistance. According to these facts, the resistance situation considered as a serious condition should be deal with it (⁴). The scientists reach to advance level of the invention of the new anticancer drug in which its target specific gene or interfere with RNA by forming (RNAi), these studies have been expanded (⁵).

These therapies include

1. Target kinase enzyme responsible for cell proliferation
2. Interfere with the immune system by making it more efficient against cancer cells.
3. Specializing the medications.
4. Using different techniques for targeting like drug delivery
5. Minimizing the side effects of anticancer drugs, etc (⁶).

There are different mechanisms involving in inactivation of the drug (⁷), these mechanisms include:

- Multi-drug resistance
· Inhibiting program cell death (suppression of apoptosis)
· Alteration in drug metabolism
· Epigenetic modification
· Changes in drug targets
· Enhance DNA repair
· Gene amplification that causes the resistance to the chemotherapy (Figure 1).

**Figure (1): mechanisms of anticancer resistance (7)**

**Intrinsic and Extrinsic Factors in Chemotherapy Resistance**

- Tumor Heterogeneity

Cancer cell differs from the normal cell in that cancer cell is highly proliferative cell as compared to normal cells. These dynamic conditions of cancer cells generate many heterogeneous. As a result of this heterogeneity, the response to the chemotherapy becomes varied either increase or decrease (8).

Gene instability induces a high degree of heterogeneity within cancer cells in intercellular genetic materials. miRNA, transcriptomic and proteomic heterogeneity are epigenetic factors, these factors occur due to primary genotypic variations, but may also represent different stages of the cell cycle, hierarchical cell organization, and stochastic cell alteration according to the stem cell theory of cancer (9). All of these collectively recognized alterations as intrinsic factors contribute to tumor heterogeneity while Extrinsic factors include other parameters, such as pH, hypoxia, and paracrine signaling interactions with stromal and other tumor cells (10). These factors are not constant, but products that directly and/or indirectly involved in the generation of drug resistance and poor prognosis may be reduced, modified, or increased.

**Tumor Microenvironment**

The tumor microenvironment plays a significant role in drug resistance, being the key explanation for multiple cancers’ relapse and incurability (11). Signals for tumor cell growth and survival are given by factors such as growth factor (GF), tumor cell-produced cytokines, which are called a tumor microenvironment (12).
Environment factors that mediated drug resistance (EM-DR) could be other factors involved in many soluble factors that mediated drug resistance products, these factors like vascular endothelial growth factor, basic fibroblast growth factor, stromal cell-derived factor-1 etc. (13).

Multi-Drug Resistance (MDR)

Significant problems of resistance to cancer chemotherapy due to multidrug resistance (MDR) in which the cancer cells have acquired the capacity to survive against a wide variety of anti-cancer drugs. MDR mechanism can be established by the outflow of chemotherapy drugs in addition to improvements in the absorption of drugs in these cells (14).

Increasing the release of drugs outside the cell

Drug efflux is mediated by a transporter family dependent on ATP so it’s called ATP-based transporters, this transporter involves in the transportation of several macromolecules including nutrients and other molecules across the cell membrane.

ATP-biding cassette transporters are very important transporter involve in drug resistance. ABC Family divided into three types, including

1. P-glycoprotein
2. Multi-drug Resistance-associated Protein-1

P-Glycoprotein (P-GP), the most critical form of the transporter. This transporter responsible for much medication resistance involves chemotherapy medication. Generally, its mechanism involves transfers chloride ion out of the cell. Sometimes these transporters bind to specific medication instead of chloride and move it outside the cell. If the cancer cell gains the ability to synthesis a huge amount of P-GP transporter it gains the ability of resistance toward specific types of an anticancer drug like doxorubicin, vinblastine. As a result, the cell releases the chemotherapy agent to the outside (17).

Reducing the Absorption of the drugs

Absorption of medication is a process of transfer medication from the site of administration to the bloodstream and from the bloodstream to the cells. Absorption is achieved either by simple diffusion or facilitated diffusion. If the cancer cell decreases its ability to absorb chemotherapy medication, this leads to chemotherapy resistance (18). The entry of chemotherapy into cancer cells occurs either with or against concentration gradients, the entry with concentration gradient mostly occurs with simple diffusion meanwhile the against concentration gradients is occur with active transporting (19). Another type of transporter that participates in chemotherapy resistance is called solute carrier family (SLE). Two important strategies adopted by cancer cells to gain resistance to chemotherapy, one of them either decreases the total number of this type of transporter and/or alteration in binding between anticancer drug and transporter (20, 21).

Inhibition of the Cell Death (Apoptosis Pathway Blocking)

The three critical events are regulated by programmed cell death (apoptosis). Necrosis, apoptosis or autophagy, of these cases. However, in their biological characteristics, these processes vary from each other (22). All these promote the death of the cell. Apoptosis happens, both internally and externally.
The metabolisms of chemotherapeutic agents can occur via different types of enzymes. This enzyme activity determines the concentration of the agent in both the inner and outer cells. The metabolism process achieves by specialized groups of enzymes mainly CYP-450. The metabolism process is divided into two phases first one called phase I which involves oxidation, reduction, and hydrolysis, and the second phase is called phase II which involves the conjugation of a metabolite with specific molecules such as glucuronide. Both phases plat an important role in the decrease of toxicity of many medications or loss its activity (23).

These reactions in phase I and phase II decrease the drug resistance in cancer cells by two means including

1. Reduce pro-drug activation (reducing the activity of certain enzymes)
2. Boost inactivation of the drug (increased activity of certain enzymes).

One of the essential examples in Phase I cell-managed reactions is the enzyme detoxification called cytochrome P450 (24). Resistance to chemotherapy in breast cancer occurs when cytochrome P450 activity increases, and the activation of cytochrome P450 contributes to the inactivation of docetaxel (25). Similarly, a better response to the treatment was observed along with the inhibition of the activity of this enzyme. The conjugation phase or what is commonly named phase II is achieved by the addition of especial molecules like glutathione, this phase causes an increase in the polarity of molecules and sometimes decreases its activity (26). The overproduction of glutathione and the detoxification resulting from glutathione transferases play a significant role in the resistance to many chemotherapy drugs such as alkylating agents and platinum-based anticancer drugs such as cisplatin and doxorubicin (27).

Changing the Chemotherapeutic Agents Targets

The effect of chemotherapeutic agents may have been based on modifications (modification), such as the mutations and changes in their target site’s expression levels. These types of modifications will eventually lead to drug resistance and the loss of their activity in the agent targets (28). One of the most important examples of changing the target site is the involved topoisomerase II enzyme. This enzyme is responsible for unwinding the DNA doubles helix. Topoisomerase II enzyme is targeted by doxorubicin which inhibited its activity, if cancer cells change the structure of topoisomerase II enzyme doxorubicin loss its ability to bind and inhibit enzyme activity (29).
Enhancing the DNA Repair

DNA repair was considered one of the most advanced drug-resistance mechanisms in the cancer region. The chemotherapeutic agents act to damage the cancer cells’ DNA directly or/and indirectly, so there are many pathways in these cancer cells that can repair the DNA damage. E.g. cisplatin that causes damage to DNA those results in the activation of programmed tumor cell death (apoptosis)\(^{(30)}\). Resistance to such agents occurs in cancer cells by enhancing the genetic material repair systems, such as the nucleotide excision repair system (NER) and homologous recombination repair mechanisms (RRM). Sometimes the success of chemotherapy treatment is depending on the repair system of cancer cells\(^{(31)}\). If the repair systems of cancer cell are very efficient and active and anticancer drug work by destroying its DNA structure, this lead to a loss of anticancer activity and resistance was seen\(^{(32)}\). Suppression of DNA repair systems in cancer cells may be one of the therapeutic objectives that can be accomplished in these systems by mutations and epigenetic silencing. Resistance to doxorubicin (alkylating agent) is also caused by enhancing DNA repair and alkyltransferase activity\(^{(33)}\).

Gene Amplification

Gene amplification is another drug resistance mechanism, considered to be 10% of cancer resistance, especially in leukemia. Increasing the number of target genes via the method of gene amplification induces the drug resistance as shown in leukemia while resisting methotrexate\(^{(34)}\). The cancer cells obtain chemotherapy resistance by making numerous copies of the Dihydrofolate reductase gene (which is methotrexate target enzyme). The amplification of the genes rises the copying of oncogenes per cell to several hundred folds\(^{(35)}\).

MicroRNA in Cancer Drug Resistance

MicroRNAs (miRNAs) are small nucleotide RNAs that are derived from hairpin structures with RNA. MicroRNAs are not protein synthesis code but play an important role in controlling the expression of genes. MicroRNAs regulate most protein-coding genes, including essential cancer genes, and in particular the generation of drug resistance to cancer\(^{(36)}\). There are three mechanisms involved in gene silencing with the miRNA process:

1. Breakdown of the mRNA strand into two pieces.
2. Destabilization of the mRNA through shortening of its poly (A) tail of mRNA.
3. Decrease the efficiency in the translation of the mRNA into proteins by ribosomes\(^{(37)}\).

miRNA plays an important role in all of the mechanisms of drug resistance listed above. miRNA involve in resistance mechanisms of cancer cells against chemotherapy. These mechanisms could be summarized as either enhancing sensitivity or preventing resistance toward chemotherapy\(^{(38)}\).

Conclusion

The overdose and/or unregulated antibiotic uses contribute to drug resistance against the bacteria. And the rapidly dividing cells and undergoing high mutation rates cause these bacteria’s resistant strains and thrive in the presence of such antibiotics. Human cancer cells with a high frequency of proliferation were considered genetically abnormal; hence the drug resistance may be similar. Interestingly, the studies accepted the intelligent cancer cells and cell stress tolerance of different forms. Drug tolerance to cancer is called a complex phenomenon. The combination therapy is the best option for drug resisted type of cancers.

Acknowledgment: The present review was accomplished in the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. The authors gratefully thank University of Baghdad for supporting the project.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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Efficacy of Hyaluronic Acid in Patients with Osteoarthritis of The Knee

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Abstract

Osteoarthritis (OA) of the knee is the most common chronic degenerative joint disease characterized by pain, stiffness, swelling and progressive functional limitation in elderly. Non-surgical management modalities like physical therapy, lifestyle modification and oral non-steroidal anti-inflammatory drugs, are often ineffective or do not alleviate symptoms adequately. Intra-articular corticosteroid (CS) and hyaluronic acid (HA) injections have been used for long to alleviate the symptoms of knee OA. viscosupplementation has been used as a therapeutic modality for the management of knee OA. The principle of viscosupplementation is based on the physiological properties of the hyaluronic acid (HA) in the synovial joint which helps tissue lubricate, cushion and reduce pain in the joint.

Study diagnosed forty patient of knee Osteoarthritis for both gender with age (45-70 years) and observed change and effectiveness to pre-and post of HA injection, All patients diagnosed by Orthopedists and Rheumatologists whose used X-rays were graded as stage I, II and III according to Kellgren and Lawrence scale.

All the measurements were used at the time of enrollment in the study before any injection and then measured again at the end of three months by using Western Ontario and McMaster University Osteoarthritis Index (WOMAC). All the patients before therapy were having minimum score of 72.4 & 9.045 for Mean and Standard Deviation respectively while after the therapy there score reduced to 36.2 & 4.783.

The results show improvement significant during four month under study and the effect peaks at around 8–12 weeks following administration, This supports the potential use of intra-articular HA as an effective long therapeutic option for patients with OA of the knee.

Keywords: Osteoarthritis, hyaluronic acid, hyaluronate physiochemical functions, extracellular matrix, glycosaminoglycan, Knee Intra-articular injection.

Introduction

Osteoarthritis of the knee is the most common slowly progressive chronic degenerative joint disease, characterized by varying degrees of loss of joint cartilage with local inflammation, usually affecting the elderly population. There is cartilage damage combined with a significant reduction in the viscoelastic properties of the synovial fluid and the molecular weight and concentration of the naturally occurring hyaluronic acid in synovial fluid decreases. This loss of viscoelasticity decreases the lubrication between joint surfaces and erodes the articular surfaces and is the mechanism of origin of pain in osteoarthritis. The patient presents with pain, swelling, stiffness, deformity, decreased range of motion and disability, which significantly affect the quality of life. The knee is the most common joint that is affected in the population with OA and plays an important role in weight bearing and mobility.
Treatment is aimed at reducing symptoms like reducing pain and inflammation and maintaining performance and normal movement of the joints and slowing the progression of the disease. It includes holistic therapeutic modalities including non-pharmacological measures like patient education, physical therapy with exercises to maintain range of motion and strength, lifestyle modifications such as dieting and weight reduction. The aim of this study was to assess the efficacy of HA intra-articular injections in managing osteoarthritic knee pain.

Characteristics of Hyaluronic Acid (HA)

HA is a glycosaminoglycan constituent of synovial fluid and cartilage matrix in normal joints. The properties of the synovial fluid are dependent on the concentration of HA and its molecular weight (MW); in OA, the concentration and MW of HA are decreased. The exogenous HA available for IA viscosupplementation is formulated as different MW preparations: low (range: 500,000–730,000 Da), intermediate (800,000–2,000,000 Da), and high MW (average: 6,000,000 Da) including cross-linked formulations of HA.

Its physiological properties include shock absorption, traumatic energy dissipation, protective coating of the articular cartilage surface and lubrication. In vitro, it is shown to have anti-inflammatory effects on cells and it may slow chondrocyte apoptosis in OA by binding CD44 and ICAM-1 receptors, and regulating in this way the process of cartilage matrix degradation. The concentration and the molecular weight of HA in the synovial fluid of patients with knee OA are shown to be reduced.

Study population

A total of 40 subjects were enrolled in this study in accordance to various criteria. These included gender (15 man and 25 woman), age (45–70 years) and Grade of disease by Kellgren-Lawrence (K-L) severity grade I, II and III OA of the knee. All subject collects of different city and diagnoses by specialists Doctors.

Materials and Methods

This prospective randomized study was diagnoses 40 patients (53 knees) with knee osteoarthritis who were treated with a single intra-articular injection of HA by 88mg/4ml lightly cross-linking HA (Monovisc®, Anika Therapeutics, Inc).

All patients who were diagnosed with knee OA by Orthopedists and Rheumatologists whose used X-rays were graded as stage I, II and III according to Kellgren and Lawrence scale. The injection was given at a site near the superolateral pole of patella in the suprapatellar pouch under aseptic conditions, with knee kept in 15–20 degree flexion and the patient was advised to take 1 day of rest after injection and apply ice to the area if there were any signs of inflammation.

All the measurements were used at the time of enrollment in the study before any injection and then measured again at the end of 3 months by using Western Ontario and McMaster University Osteoarthritis Index (WOMAC) was used as a self-administered test to compared as regards pain and functional improvement pre and post used the HA injection, with the using of WOMAC (Appendix 1), a lower score represented a better outcome.

WOMAC

The WOMAC is a self-administered questionnaire that is composed of 24 questions categorized into three subscales (pain, stiffness, and physical function). In the current study, we used 5-point Likert scale format: none (0), mild (1), moderate (2), severe (3), and extreme (4). Scores for each subscale were determined by summing the component item scores for each subscale—possible score range: pain (0–20), stiffness (0–8), and physical function (0–68). The final total scores (possible score range, 0–96) were determined by summing the scores for each subscale. WOMAC-total score in the range of 0 (best score) to 96 (worst score).

Results

Forty patients enrolled in our study suffered from osteoarthritis, who were treated with HA intra-articular injections, Pain and stiffness and physical function on Knee patients were estimated pre and post treatment by used WOMAC index.

The subjective scoring with WOMAC score done after 4 months showed gradual decrease in WOMAC score which suggests that the patients have observed
relief in pain, stiffness and physical has improved due to importance of hyaluronic acid (HA) and its physiological and biochemical functions at cellular level (Table 1). All the patients before therapy were having minimum score of 72.4 & 9.045 for Mean and Standard Deviation respectively while Significant improvement (p<0.05) were obtained After the therapy there score reduced to 36.2 & 4.783 (Figure1).

And based on individual results for each patient, it was observed that best effectiveness of the HA intra-articular injections was for patients suffering from primary knee OA with Kellgren and Lawrence (KL) Grade I and II, while in patients suffering advanced OA in last grade III of Kellgren-Lawrence, it was observed that were less effective.

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>Pre Therapy</th>
<th></th>
<th>Post Therapy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Pain</td>
<td>15.2</td>
<td>2.315</td>
<td>4.8</td>
<td>1.720</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>51.2</td>
<td>5.635</td>
<td>27.2</td>
<td>2.315</td>
</tr>
<tr>
<td>Stiffness</td>
<td>6</td>
<td>1.095</td>
<td>4.2</td>
<td>0.748</td>
</tr>
<tr>
<td>Total WOMAC Score</td>
<td>72.4</td>
<td>9.045</td>
<td>36.2</td>
<td>4.783</td>
</tr>
</tbody>
</table>

Table 1. WOMAC Index Pre and Post treatment.

Figure 1. Comparison of Mean WOMAC values in Study Group Pre and Post treatment by HA injection.
**Discussion**

Primary treatment goals in knee OA include pain reduction and improvement of joint mobility and function. The secondary goal is to decrease the progression of disease. Vissco-supplementation with hyaluronic acid is a non-operative intervention.

WOMAC Scores showed improvement in all the parameters post-intervention after 4 months, there is due to it keeps the space between your bones well lubricated and viscoelasticity of the synovial fluid. When the joints are lubricated, the bones are less likely to grind against each other and cause reduce pain.

Moreover hyaluronic acid supplements are very helpful for people suffering from osteoarthritis, a type of degenerative joint disease caused by wear and tear on the joints over time. whereas high-impact movements that generate compressive forces on a healthy joint benefit from the shock absorption properties of HA. Furthermore, with inflammation, HA may offer protective properties to the joint tissue by scavenging free radicals and reducing oxidative damage.

Injected HA’s main functions stem from its antinociceptive properties, ability to improve the viscoelastic properties of synovial fluid, and indirect lubrication of the joint through stimulated endogenous HA synthesis. Other potential anecdotal benefits of HA injections that have been proposed include chondroprotection and potential function as a disease-modifying agent for OA. More specifically, Type II collagen degradation products appear to be a marker of cartilage degradation and OA disease activity.\(^{15}\)

The improvement was more significant in Hyaluronic acid at the end of 4 months providing a good cue to compare the clinical efficacy before and after intervention over four months.

**Conclusion**

According to results, the intra-articular HA injection was very effective in pain relief and improvement of knee function beginning from three months.

There is good evidence for the efficacy of HA injection in reducing pain and increasing function in knee OA. this supports the potential use of intra-articular HA as an effective long therapeutic option for patients with OA of the knee and we recommends that use of HA injection in knee OA patients with mild to moderate disease to get best result.

**Conflict of Interests:** Nil.

**Ethical Clearance:** Take from AL-Hussein Teaching Hospital by approval ethical committee.

**Funding:** Self-funding.

**References**


Changes in Interleukins and Follicle Stimulating Hormone in Toxoplasmosis Male Patients

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Abstract

In several countries there are endemic of parasitic diseases, specifically in developing countries, several studies investigated that toxoplasmosis is related with sterility of male. Aims of study are assessment of anti-Toxoplasma IgG antibodies in patient’s serum and their relation with interleukins and FSH hormone. IL-6 stimulates antibody production and extend effect of proinflammatory by stimulating the production of acute phase proteins. IL-10 and IL-12 control the immune response type. Cytokine synthesis is inhibits by the former and by blocking the IL-6 and TNF-α production, the response with involvement of Th2 and activation of B cells has an advantage. Detection for the toxoplasmosis which is based on toxo IgG , interleukins and FSH used the classic ELISA technique that based on immunoassay system using fluorescence technology and antigen antibody interaction. They were found in 1 (3.03%) of azoospermic patients and 4(12.12%) of oligozoospermic patients, while the negative results found in 32(96.97%) of azoospermic patients and 29(87.88%) of oligozoospermic patients. The control group had 34(100%) negative results in toxoplasmosis. The mean serum levels of IL-6 and IL-17 (pg / ml) increased significantly in patients with Toxoplasma gondii compared to the control group (237.97±9.09 pg / ml 285.52±12.45and pg / ml, respectively; p<0.0001) . In this study, it has been concluded that infection with T. gondii can effect on the level of the interleukins and FSH in infertile couples.

Keyword: Toxoplasma gondii, Interluekin, FSH hormone , Cytokine , Immune Response

Introduction

Toxoplasmosis is caused by parasite Toxoplasma gondii, then might be cause impaired folliculogenesis , endometritis, uterine and ovarian atrophy, vasculitis ,adrenal hypertrophy and decrease in semen quality, concentration, and motility in male (1). Through ingestion of parasite cysts present in infected foods, undercooked or through swallowing oocysts contained in polluted water and vegetables it is highly transmitted. So, human infections from T. Gondii are normal but are minimally symptomatic or asymptomatic in most cases of immunocompetent humans and their effects may remain unnoticed (2) . It was confirmed that in this case, T. Gondii infection is slightly higher in infertile couples relative to fertile couples. Another researchs found that the amount of anti-sperm antibody in toxoplasma infected pairs was higher than in non-infected pairs (3) . For most cases of adults infection with T. Gondii doesn’t cause severe illness, but during pregnancy may have implications for the deformities of developing fetus (4). It was later reported that after the T. gondii infection there was a reduced fertility in the rats. along with lower epidididymis weight, and increased irregular morphology of the sperm. In addition, the association between increased apoptosis of sperm and toxoplasmosis, especially of diploid spermatozoa (5).

Cytokines are significant mediators during the whole pregnancy in the bidirectional association between the reproductive system and the maternal immune system (6) . IL-17 is a pro-inflammatory cytokine created by several tissues induced by several factors, inclusive viruses and different cytokines, whose effect is directed cellular targets either to myeloid or non-myeloid (7) . Various cytokines (IL-4, IL-5, IL-6, IL-10, IL-13, and IL-14)
synthesize by Th2 lymphocytes, which play an important part in pathogenesis of parasites. IL-6 stimulates the synthesis of antibodies and extend proinflammatory action by stimulating acute phase protein development (8). Both IL-10 and IL-12 regulate the form of immune response. Cytokine synthesis is inhibits by the former and by blocking the IL-6 and TNF-α production, the response with involvement of Th2 and activation of B cells has an advantage, reaction with Th2 involvement and B cell activation has an advantage (9).

Materials and Methods

This study was conducted on 130 samples (100 Patients , and 30 control group ) their age ranged from (19-55years). Blood samples were collected from High institute of infertility diagnosis during the period from December to March 2020. About 5 ml fasting venous blood specimens were gathered from both patients and control groups , and transferred into anticoagulant gel tube for centrifugation. After that, serum was separated which then used for assess biochemical parameters.

Follicle-stimulating hormone (FSH) was evaluated by (1-chromaTM FSH), and IgG was assessed from serum samples by enzyme-linked immunosorbent assay (ELISA) using Human IgG anti Toxoplasma gondii ELISA Kit (Human company).

The levels of interleukins were assessed in blood serum using ELISA method for IL-6, and IL-17 by (MyBioSource, USA, Cat No. MBS261259, and Cat No MBS764076) respectively.

Results

Table (1) show the presence of anti-toxoplasma gondii IgG antibodies (ELISA test) in 100 men. They were found in 1 (3.03%) of azoospermic patients and 4(12.12%) of oligozoospermic patients, while the negative results found in 32(96.97%) of azoospermic patients and 29(87.88%) of oligozoospermic patients. The control group had 34(100%) negative results in toxoplasmosis.

Table (1). Distribution of samples study according to presence of anti- toxoplasma IgG antibodies with difference groups.

<table>
<thead>
<tr>
<th>The Groups</th>
<th>Positive No. (%)</th>
<th>Negative No. (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia (No. = 33)</td>
<td>1 (3.03%)</td>
<td>32 (96.97%)</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Oligozoospermia (No. = 33)</td>
<td>4 (12.12%)</td>
<td>29 (87.88%)</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Normozoospermia (No. = 34)</td>
<td>0 (0.00%)</td>
<td>34 (100%)</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>P-value</td>
<td>0.00965 **</td>
<td>0.00965 **</td>
<td>---</td>
</tr>
</tbody>
</table>

** (P<0.01).

When study the hormonal change of toxoplasmosis infected patients can observe low testosterone level in 1 (3.03%) of azoospermic patient and oligozoospermic patient, while there were decreased in FSH and LH hormones in 2 (6.06%) of oligozoospermic patients as it clear in table (2).
Table (2): The hormonal study of toxoplasmosis infected patients.

<table>
<thead>
<tr>
<th>The Groups</th>
<th>FSH (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=33) Azoospermia</td>
<td>0(0.00%)</td>
</tr>
<tr>
<td>(n=33) Oligozoospermia</td>
<td>2(6.06%)</td>
</tr>
</tbody>
</table>

The mean serum levels of IL-6 and IL-17 (pg / ml) increased significantly in patients with Toxoplasma gondii compared to the control group (237.97±9.09 pg / ml 285.52±12.45and pg / ml, respectively; p<0.0001) as shown in Table 3.

Table (3). Laboratory results of patients and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample</th>
<th>No.</th>
<th>Mean</th>
<th>Standard Error of Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6(pg/ml)</td>
<td>Control</td>
<td>30</td>
<td>85.32</td>
<td>11.26</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>100</td>
<td>237.97</td>
<td>9.09</td>
<td></td>
</tr>
<tr>
<td>IL-17(pg/ml)</td>
<td>control</td>
<td>30</td>
<td>41.94</td>
<td>3.22</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>patient</td>
<td>100</td>
<td>285.52</td>
<td>12.45</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The one of most usual disease consider to have adverse effect on the human reproduction is toxoplasmosis; previous studies of parasite infections in the male genital tract proved the possibility of *T. gondii* infection increase the defects of testicular during secondary hypogonadism via hypothalamic-hypophyseal axis changes and found that toxoplasmosis can change all spermatic parameters include concentration, morphology and motility that lead to the production of significantly decreased sperm cells but without sterility (10).

This study proved that the infection with *T. gondii* in infertile male is intensity higher than fertile male, four patients of oligozoospermic patients and one patient of azoospermic patients have toxoplasmosis infection.

The patients of *T. Gondii* had significantly higher the IL-6 and IL-17 levels compared to the control group, which indicate an inflammatory condition. The key role of IL-6 is immune response involvement by lymphocyte intervention B. It is a mediator in charge of the development of acute phase protein and the cytotoxic activity of NK cells will be increased, though unspecific, inflammatory marker which states this study agreement with Matowicka’s study found *T. Gondii* patients had double the IL-6 levels compared with control group (11).

The study conducted by Satti showed the mean serum level of IL-17 in patients a highly significant increase when compared with control group (12) this agreement with our study. In the present study, the early increase in IL-17 serum level matches the results of different researchers (13), investigated that an early rise in IL-17 was identified in the early stages of infection. IL-17 was also presented to be included in the early recruitment and development of neutrophils, which are necessary for parasite clearance during initial stages of infection (14).

Previous studies correlated the decrease in the concentration of sperms with toxoplasmosis infection that *Toxoplasma* parasites lead to elevate the level of cell apoptosis that is stimulated by a decreased in
gonadotropin level. As well as, decrease in the levels of LH, FSH and testosterone and these results effect the spermatogenesis process that lead to decrease proliferation of sperms and spermatocytes which predict to be the most sensitive stage (15). Marathe’s research showed that, in addition to testosterone and FSH, selective immunoneutralization of LH triggers the apoptotic cell death of meiotic and post-meiotic germ cells in testes that have been shown to regulate the survival of germ cells, the cellular apoptosis in the testis result from exposure to decrease of hormones which related with the lower numbers of sperms in toxoplasmosis infected patients. Also more previous findings indicated that Toxoplasma has the effect on the efficiency of immune system and induction of IL-1b which triggered the modification of hypothalamic GnRH release and may be manufacture is interposed by increased release of the dopamine and norepinephrine from neurons in the brain stem as well as, hypothalamus that are suppressor to gonadotropin releasing hormone (16). Another studies indicated that cytokines have more effects in deficiency the release of anterior pituitary hormones. Also, cytokines are show to depress the HPG axis, directly or indirectly via increased corticotrophin-releasing hormone (CRH) and/or cortisol. indicating it or not changed CRH and/or cortisol participate in part to HPG axis selectively suppress serum FSH concentrations (17).

**Conclusion**

Toxoplasmosis infection leads to decrease in sperm concentration. As well as, significantly increased the levels of interleukin in contrast, decrease in the level of FSH of infected patients affect the spermatogenesis process that lead to decrease proliferation of sperms and spermatocytes in toxoplasmosis patients.

**Acknowledgement:** Thanks to all the staff of laboratory in the High institute of infertility diagnosis and assisted reproductive technologies in Baghdad, Iraq.

**Ethical Clearance:** Taken from University of Kufa ethical committee

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Evaluation of the Acute Flaccid Paralysis Surveillance System of Polio Free in East Java, Indonesia, 2019

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Abstract

Background: Proving that the wild polio virus no longer exists in Indonesia, the symptoms must be found that resemble polio. These symptoms are found in patients with AFP (Acute Flaccid Paralysis). AFP surveillance is an observation made of all cases of acute paralysis or AFP in children aged <15 years who are vulnerable to polio. The priority problem that is still found in the East Java Provincial Health Office regarding AFP surveillance is the reduction in the discovery of non-polio AFP cases and adequate specimens.

Aims: The purpose of this study was to analyze the implementation of AFP surveillance activities in the East Java Province Health Office in 2019.

Methods: Data collection was carried out by primary and secondary to study the AFP surveillance overview. Primary data were obtained from in depth interviews with AFP surveillance program holders in East Java Provincial Health Office. Secondary data is processed using Microsoft Office Excel, Epi Info and Quantum GIS.

Results: The result is in 2019 the rate of non-polio AFP and the percentage of adequate specimens in East Java Province has under the target. In 2019 there was a decrease compared to the previous year, the non-polio AFP rate was 1.64 and the percentage of adequate specimens was 56.2%. Only as many as six districts / cities in East Java Province had pass these two indicators.

Conclusion: The lack of visits and reports from both the hospital and the community is one of causative factor. Intensive and routine AFP surveillance is needed to monitor the emergence of polio cases

Keywords: Keywords: AFP, surveillance, polio, adequate specimens.

Introduction

Poliomyelitis is an infectious disease caused by the poliovirus. Polio virus predilection can cause paralysis, muscle atrophy and even irreversible paralysis to death in children. Since this disease causes paralysis, polio is one of the important diseases to be eradicated globally(1). The polio eradication program was launched by the World Health Organization (WHO), known as the Global Polio Eradication Initiative (GPEI). GPEI in Indonesia is known as ‘Eradikasi Polio’ (Erapo) or Polio Eradication which aims to break the chain of transmission of the polio virus globally through coordinated efforts nationally and internationally. The program is based on evidence that humans are the only reservoir of the polio virus(2). Poliomyelitis cases are mostly non-paralytic or not accompanied by clear clinical manifestations. A small portion (1%) of cases of poliomyelitis that causes paralysis (Paralytic Poliomyelitis) requires observations focused on cases of poliomyelitis through AFP surveillance (Acute Flaccid Paralysis)(3).

AFP (Acute Flaccid Paralysis) surveillance is an observation made of all cases of acute paralyzed paralysis or AFP (Acute Flaccid Paralysis) in children aged <15 years who are vulnerable to polio. In 2005 there was an
outbreak of polio which resulted in an increase in public awareness of all the paralysis that occurred, so that the discovery of AFP cases instead of polio increased by more than 2/100,000 even though the specimen was adequately less than 80%. The discovery of this case shows a minimum estimate of non-polio AFP cases in Indonesia. Based on this, since 2006 non-polio AFP rate was set 2/100.000 children aged less than 15 years(3).

The problems that are still found in the East Java Provincial Health Office regarding AFP surveillance are:

a. The reduced discovery of the non-polio AFP case

b. Decreased percentage of adequate specimens (Target ≥80%)

c. 60 days return visits is under the target

d. The percentage of completeness of the AFP report is still low

Based on four problems above, the East Java Provincial Health Office determines two issues that are priority and important to study in order to obtain an evaluation in assessing the performance of AFP surveillance in East Java Province. The decrease in finding of non-polio AFP cases and adequate specimens are a priority issue in AFP surveillance.

**Material and Methods**

This study was sourced from the East Java Provincial Health Office. Secondary data collection was obtained from AFP surveillance performance reports that have been processed by the East Java Provincial Health Office. All data is processed using Microsoft Office Excel and Epi Info to determine the distribution of cases based on time, place, and person. In addition, secondary data processing is used to determine the percentage of AFP surveillance performance that pass the target or under the target. Distribution of non-polio AFP cases by place in all districts/cities of East Java Province is described using the Quantum GIS application. Quantum GIS is an open source software that can be used for spatial data processing and geographic information system application development, so that the data used is data that has coordinates or location instructions internationally namely longitude and latitude(4). Primary data collection to study the surveillance overview and evaluation was obtained from in-depth interviews with AFP surveillance program holders and surveillance coordinators in the East Java Provincial Health Office. In addition, a study of Minister of Health Decree document No. 483 of 2007 was conducted.

**Results**

**Overview of AFP Surveillance Recording and Reporting System**

1. Collecting Data

The earliest implementing units in the AFP case finding were Puskesmas (Primary Health Care) and Hospitals in the sub-district, district/city areas. Primary health care and local hospitals reported cases as sources of AFP surveillance reports. Reporting no later than the 10th of every month. The AFP case is cumulative and will be identified based on AFP surveillance performance. The District/City Health Office also sends the FP1 form (AFP Case Tracking Form) to the East Java Provincial Health Office.

2. Data Processing

Data obtained from the District/City Health Office is collected and processed using Ms. Office Excel and Epi Info by the East Java Provincial Health Office. The purpose of the grouping is to facilitate data analysis according to epidemiological variables, namely people, place, and time.

3. Data Analysis

The East Java Provincial Health Office conducted an analysis of AFP case data using the Ms Office Excel and Epi Info applications. These results are also presented in the form of AFP surveillance performance. A spatial analysis was also made about the distribution of non-polio AFP cases and adequate specimens and classified according to good, moderate, poor and very poor categories.

4. Data Dissemination

The East Java Provincial Health Office follows up or give feeds back to the District/City Health Office every three months by sending back AFP case data, weekly report attendance, and AFP surveillance performance analysis. It is aims to make District Health Office to check the data and immediately report if it has been not
Benefits of AFP surveillance

The benefit of AFP surveillance is the data collected can detect at least 1 (one) AFP case among 100,000 children aged <15 years. A very strong AFP surveillance system is very important to detect early wild poliovirus as the ultimate goal of eradicating polio(5).

Analysis Results

Secondary data obtained will be processed to determine the distribution of cases based on time, people, and place variables.

a. The distribution of non-polio AFP cases by age group in East Java in 2016-2019 is as follows:


Figure 1 shows the distribution of non-polio AFP cases by age group. Age of children is grouped into five categories, namely the age group <1 year, age 1-4 years, age 5-9 years, age 10-14 years, and age ≥15 years. The case of non-polio AFP in East Java Province throughout 2016-2019 tends to attack the 1-4 years and 5-9 years age groups.

b. The distribution of non-polio AFP rates in 2014-2019 are as follows:

The target of non-polio AFP rate for people aged <15 years is ≥ 2/100,000. The non-polio AFP rate from 2014 to 2019 tends to fluctuate.

c. The percentage distribution of adequate specimens for 2014-2019 is as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Adequate Specimens Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>84.3%</td>
</tr>
<tr>
<td>2015</td>
<td>90.5%</td>
</tr>
<tr>
<td>2016</td>
<td>88.3%</td>
</tr>
<tr>
<td>2017</td>
<td>94.3%</td>
</tr>
<tr>
<td>2018</td>
<td>56.3%</td>
</tr>
<tr>
<td>2019</td>
<td>56.2%</td>
</tr>
</tbody>
</table>


The target specimen examination results received from the laboratory within ≤ 14 days is ≥ 80%. From 2014 to 2017 the percentage of adequate specimens passed the target of ≥80%. But in 2018 and 2019 the percentage of adequate specimens has under the target, the percentage <80%.

d. Distribution of non-polio AFP rates based on districts/cities in East Java Province in 2019:


Districts/cities with case finding rates ≥2/100.00 are depicted with a map of the area in green and have been categorized well. Districts/cities with case finding rates <2/100,000 are depicted on a red map and are categorized as poor.
e. The percentages distribution of adequate specimens by districts/cities in East Java Province in 2019:

**MAP DISTRIBUTION OF ADEQUATE SPECIMENS PERCENTAGE BASED ON AFP SURVEILLANCE PERFORMANCE IN EAST JAVA 2019**

![Map showing distribution of adequate specimens percentage in East Java Province in 2019](image)

Figure 5 shows the percentage of good adequate specimens ≥80% on the map above marked with a green zone. A total of 15 districts/cities are categorized well in the percentage of adequate specimens. As many as 23 districts/cities still lack adequate specimens.

**Discussion**

**AFP and Non-Polio Cases of East Java Provincial Health Office**

a. Distribution of Non-Polio AFP Cases by Age Group in 2016-2019

Figure 1 shows the distribution of non-polio AFP cases by age group in the East Java Provincial Health Office in 2016 to 2019. The results show that the distribution of cases most occurred in the 1-4 year age group and 5-9 year age group. These results are consistent with research by Soltani et al that most AFP cases occur in the 0-5 years group(6). Research conducted by Momen and Shakurnia (2016) in Iran also shows that more than half of non-polio AFP cases occur in the age group under five years(7).

b. Distribution of adequate specimen percentage for 2014-2019

Specimens sent to the laboratory and arrive at the laboratory are in eligible conditions with a target of ≥80%. Adequate specimen examination which includes examination of stool can contain poliovirus. The polio virus in feces is not only found in children who have just been immunized but in children who are not immunized but are infected with the polio virus through the oral faecal of immunized children(9). Figure 3 shows that the percentage of adequate specimens in 2014 to 2017 has been more than 80%. But in 2018 and 2019 the percentage of adequate specimens is less than 80%.

c. Distribution of Non-Polio AFP Rate by Districts/Cities in East Java Province in 2019

Based on AFP surveillance data from the East Java Provincial Health Office, the distribution of non-polio AFP rates exceeded the target (≥2/100,000) occurred in 2014, 2017 and 2018. In 2019 the non-polio AFP rate has under the target. Research conducted by Dhiman et al in India shows that the rate of AFP not polio in 2004 to 2017 greatly exceeded the target (≥2/100,000)(8).
Figure 4 shows the distribution of non-polio AFP rates per district/city in the East Java Provincial Health Office. A total of 25 districts/cities were declared bad in the non-polio AFP rate in 2019. 13 regencies/cities were declared good in the non-polio AFP rate, which pass the target of ≥2/100,000.

e. Distribution of Adequate Specimens by Districts/Cities in East Java Province in 2019

Specimens from the AFP case were categorized as good if at least two stool specimens were obtained within 24 hours apart within 14 days after the discovery. The specimen is then sent to a WHO accredited laboratory, and the specimen must be in good condition(10). The quality of AFP surveillance is conventionally evaluated, one of which is collecting adequate faecal specimens. Collection of specimens in a number of areas requires collection of kits. If it is not available it becomes very difficult to assess the presence of a virus(11).

Distribution of AFP cases by Districts/cities based on the performance of AFP Surveillance in 2019

East Java Provincial Health Office classifies districts/cities in several categories. The category is good if the Non-polio AFP rate is ≥2 and the specimen is adequate ≥80%. The category is moderate if the Non-polio AFP rate is ≥2 and the specimen is adequate <80%. The category is less if the Non-polio AFP rate <2 and the specimen is adequate ≥80%. The category is very less if the Non-polio AFP rate <2 and adequate specimens <80%.

MAP DISTRIBUTION OF AFP CASES BASED ON AFP SURVEILLANCE PERFORMANCE IN EAST JAVA 2019

Legend

\[
\begin{array}{c}
\text{Good, non AFP rate} \geq 2 \text{ and adequate specimens} \geq 80 \\
\text{Less, Non-polio AFP rate} < 2 \text{ and adequate specimens} \geq 80\% \\
\text{Very less, Non-polio AFP rate} < 2 \text{ and adequate specimens} < 80\% \\
\text{Moderate, Non-polio AFP rate is} \geq 2 \text{ and adequate specimens} < 80\%
\end{array}
\]

Figure 6. Map Distribution of AFP Cases Based on AFP Surveillance Performance in East Java 2019.

Source: AFP Surveillance Report on East Java Provincial Health Office, 2019

Figure 6 shows that 16 districts are still very lacking, marked by a red zone with a non-Polio AFP rate <2 and an adequate specimen <80%. A total of eight districts are categorized moderate with non-polio AFP rate ≥2 and adequate specimens <80%. A total of six districts/cities have been declared good, with marked green zones, non AFP rate ≥2 and adequate specimens ≥80. Districts that have been declared good are Sumenep, Surabaya, Madiun, Pacitan, Blitar, and Tulungagung.

The AFP non-polio AFP rate and the percentage of adequate specimens that exceed the target is very important in assessing AFP surveillance performance. The non-polio AFP rate has been proven in administering doses of polio vaccine. This can be causatively related to OPV vaccination(8).
Factors Causing the Reduction in AFP Case Discovery and Adequate Specimens

Weaknesses found in the conduct of AFP surveillance in the East Java Provincial Health Office include the lack of socialization, training and development for AFP surveillance officers in primary health care, hospitals, and district/city health offices. The one of effectiveness in AFP surveillance depends on the community reporting a disease. The lack of socialization about the AFP case to the communities also becomes a weakness in conducting AFP surveillance.

Conclusion

To prove that the wild polio virus no longer exists in Indonesia, symptoms must be found that resembles polio. These symptoms are found in patients with AFP. The non-polio AFP rate in East Java Province in 2019 is 1.64 (target ≥2/100,000). All specimens for virus isolation must be collected as soon as possible after symptoms appearance. In 2019, most districts/cities have adequate specimen which is not reaching 80%.

The East Java Provincial Health Office classified districts/cities into several categories based on non-polio AFP indicator and the percentage of adequate specimens. The results showed that 16 districts are still said to be very lacking. A total of eight districts have been said to be moderate, and six districts/cities have been declared good. AFP surveillance must be carried out intensively and routinely to produce good surveillance performance in finding AFP cases as indicators of polio eradication.

Acknowledgements:

a. Improved implementation of HRR (Hospital Record Review) by the East Java Provincial Health Office optimally by increasing coordination with stakeholders.

b. Supervision by the East Java Provincial Health Office can be carried out routinely and optimally to evaluate the achievement of performance indicators for each District/City Health Office. Implementation of supervision can also improve coordination between relevant parties.

c. Increased collaboration with local governments, hospitals, health centers, in an effort to strengthen commitment and support for the implementation of AFP Surveillance.

Ethical Approval: Not applicable.

Source of Funding: None.

Conflict of Interest Disclosures : There is no conflict of interests.

References


E-Cigarette Vaping and Periodontium: A Systematic Review

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Abstract

Conventional cigarettes have shown severe toxicity on immune cells and wound healing in the periodontium, but little is known about the comparative effects of vaping or electronic cigarettes. If current conventional cigarette users are to transition to a less detrimental alternative, the evidence must demonstrate if electronic nicotine delivery systems can be deemed safer than conventional options. The intent of this literature is to summarize the evidences on the effect of E-cigarette on the periodontal health.

The main sources of evidence for this report included: a comprehensive report from National Academies of Sciences, Engineering, and Medicine, reviews published in the past 10 years.

As a conclusion, further investigation and studies are needed to evidence the effect of new smoking methods on the oral health of newly users and on the shifted persons who was previously tobacco smokers.

Keywords : E-cigarettes, Vaping, Periodontium, Nicotine, Aerosols

Introduction

In recent years, electronic e-cigarettes have been gaining popularity. The main purpose of invention of e-cigarettes was to replace the conventional cigarette for helping in smoking cessation (1). E-cigarettes are the electronic devices that produce aerosol. Aerosol is a mixture of fine particles/liquid droplets suspended in a gaseous medium (2).

Several authors have shown that tobacco smoking, was a risk factor in periodontal disease progression, and can also impair the effectiveness of periodontal treatment. Over the past years, a new mode of nicotine delivery has been introduced, claiming less harm to the consumer when compared to conventional smoking (3). Electronic-cigarettes (e-cigs) represent a significant and increasing proportion of tobacco product consumption, which may pose an oral health concern. Different level evidence including systematic reviews of randomized control trials (RCTs) on the effect of E-cigs on the periodontium is currently lacking and the available data worldwide is necessary to allow further knowledge on this subject (3).

E-Cigarettes

E-cigarettes or Electronic nicotine delivery systems (ENDS) are battery-operated devices that electronically heat a solution to create an inhalable aerosol. This solution, also known as ‘e-liquid’ or ‘e-juice’, is commonly made up of propylene glycol or glycerin, water, flavorings and nicotine (although many liquids do not contain nicotine) (4). E-cigs are battery operated devices, which consist of a metal heating element in a stainless steel shell, a cartridge, an atomizer and a battery. The heating element vaporizes a solution containing a mixture of chemicals including nicotine and other additives/humectants, such as base/carrying agent’s propylene glycol, glycerin/glycerol, and flavoring agents including fruit and...
candy flavors. Apart from inhaled nicotine, variable levels of aldehydes and carbonyls are detected in e-cig aerosols during vaporizations (5,6). E-cigarettes are also commonly referred to as ‘vape pens’, ‘hookah pens’ or ‘e-hookah’ among youth (7,8).

**History of evolution**

Electronic smoking devices are not a new phenomenon, with a patent being recorded in 1965 in the USA (9). The modern rise of e-cigarettes is attributed to Hon Lik, a Chinese inventor, who filed a US patent in 2005 for an ‘electronic atomization cigarette that contains nicotine without tar’ (10). E-cigarette devices firstly introduced in the early 2000s (11). Over the years, e-cigarettes have evolved to more complex devices that come in different shapes and sizes, also involved changes in device configuration and components (12).

Newer versions of e-cigarettes, such as JUUL pods, incorporate nicotine salts in a novel product design. These more recently available products have a higher nicotine content, have become immensely popular with users, particularly among youth, and account for a major portion of the e-cigarette market share in the US at this time (13).

**Types of E-Cigarettes**

**First generation**: Also known as ciga-likes/minis. It is low cost and disposable. 200-300 puffs per cartridge which is equivalent to 1 pack of 20 cigarettes. It has limited battery life. The nicotine delivery is poor.

**Second generation**: Also known as ‘tanks’. It resembles a pen. It contains a tank which is refillable. It has larger batteries and is rechargeable.

**Third generation**: Also known as ‘mods’. It has advanced features like adjustable voltage systems and is digitalized (14).

**Content of fluid and vapor**

Most studies have used conventional cigarettes as reference and investigated presence or concentrations of substances that are known to be harmful in conventional cigarettes. Some of the studies performed in vitro experiments with cells exposed to fluid or vapour, for example to test for cytotoxicity or viral defense (15). These studies are also mentioned in this section. Many studies found that the product labels did not show the ingredients (e.g. flavours, solvent, nicotine) or that the declaration did not correspond with the concentrations found (e.g. of nicotine) (15). The following ingredients may be found:

1. **Glycols.**
2. **Nicotine.**
3. **Particles.** One study found that e-cigarette liquids generate many nanoparticles, up to 3000 times more than found in ambient air (16).
4. **Metals.** Such as lead and chromium, nickel, tin, silver and aluminum (17).
5. **Tobacco-specific nitrosamines.**
6. **Carbonyls.**
7. **Volatile organic compounds.**
8. **Hydrocarbons and polycyclic aromatic hydrocarbons.**
9. **Phenols** (15).

**Effects of smoking on periodontal diseases**

Epidemiological studies have demonstrated that tobacco use is a significant risk factor for the development of periodontal diseases (14). Disease severity increases with the frequency of smoking (18). Smokers accumulate markedly more dental calculus than do non-smokers, and the quantity of calculus is correlated with the frequency of smoking (14). Smoking is also associated with an increased risk of periodontal attachment loss and formation of periodontal pockets, as well as alveolar bone loss.

The adverse effects of smoking on the periodontium correlates well with both the quantity of daily consumption and the duration (19). Approximately half of the cases of periodontitis in the United States have been attributed to smoking (20). Smokers were recorded to have a 2.5 to 3.5 times greater risk of severe periodontal attachment loss (14).

**Mechanism of the effect of tobacco on the periodontal tissue**:
The main property of nicotine is the vasoconstrictive effect at the end-arterial vasculature of the gingivae and other tobacco components can also induce tissue necrosis and ulceration seen in the disease. Smokeless tobacco users known to possess a painless loss of gingival tissues and alveolar bone destruction in the area of chronic tobacco contact, as a result of collagen breakdown due to increased release of collagenase (22). Nicotine inhibits the growth of gingival fibroblasts and their production of fibronectin and collagen (23). Furthermore, oral leukocytes, especially neutrophils, may exhibit diminished ability to migrate and phagocytose, and they contribute to the inactivation of tissue protease inhibitors (23). Tobacco smoking may exert a masking effect on gingival symptoms of inflammation, which might give smoking patients a false sense of assurance of gingival health (22). Interleukin-1 genotype positive smokers are more susceptible to severe adult periodontitis (24).

**Effect of e-cigarette on periodontal tissue**

Since e-cigarettes became available in the markets, their safety and use as a substitute for tobacco smoking have been surrounded by medical and public controversy. However, a recent report by the Royal College of Physicians concluded that e-cigarettes are likely to be much safer than smoking (25).

The effect of e-cigarette use on the gingival condition and inflammatory biomarkers has not yet been investigated (26). E-cigarette users also have been known to develop lacy white patches on the gums, tongue or insides of the cheeks, a condition called an oral lichenoid reaction. Oral thrush also can develop in vapers, caused by an overgrowth of Candida yeast in the mouth (27).

It could cause a pretty significant burn, where you lose at least the top layer of skin, if not more. Device explosions also have caused people to lose teeth, the findings showed. For example, an estimated 2,035 people with electronic cigarette burn injuries were treated in U.S. emergency rooms between 2015 and 2017, more than 40 times the number of vaping burns reported between 2009 and 2015, researchers found (28).

**Nicotine and periodontal health**

Nicotine is vasoconstrictor. It constricts the arteries and reduces the amount of oxygenated blood flow and nutrients to the gums thereby reducing the wbc’s that provide anti-inflammatory action against harmful foreign substances. Three types of bacteria thrive in a deoxygenated environment which are porphyromonas gingivalis, aggregatibacter actinomyctem comitans, prevotella intermedia (29). These bacteria are found in plaque, tartar and pocket depth of up to 3mm. This leads to the destruction of PDL and alveolar bone. Without sufficient blood gingival and periodontal tissues cannot be healthy. Also nicotine is a muscle stimulant which causes bruxism due to hyperactivity of the muscles (30).

Cigarette smoke induces epidermal growth factor receptor-dependent redistribution of apical Mucin 1 and junctional beta-catenin in polarized human airway epithelial cells (31). Desmoglein 3 and keratin 10 expressions are reduced by chronic exposure to cigarette smoke in human keratinized oral mucosa explants (32).

**Aerosols and periodontal health:**

E-cigarettes produce aerosol. Aerosol can cause carbonyl inflammatory action. Also heat produced from the vapor leads to xerostomia . Saliva contains lysozymes that kill the bacteria. Therefore, xerostomia leads to formation of harbor for the bacteria to pool in. Especially in the advanced featured cigarettes the voltage is regulated at high temperature for more effect which causes xerostomia. When e-cigarettes are heated to high temperature it produces carbonyls like formaldehyde, acetaldehyde. Researchers have noted that Propylene based e-liquids produced more carbonyls. There were multiple user questionnaires/surveys that repeatedly detailed ‘mouth and throat dryness and irritation’ as one of the most common reported side effects of e-cigarette use (31,33).

An in vitro study on periodontal ligament fibroblasts demonstrated decreased fibroblast proliferation rates with menthol additives (31). The topical effects of nicotine are worthy of specific consideration. The nicotine in the aerosol is primarily absorbed in the buccal and pharyngeal mucosa, rather than the alveoli, demonstrating the potential to have effects on the oral tissues (34).
Conclusion

Numerous studies over the past several years have made stronger correlations to the effects of conventional cigarette smoking on the periodontium. A systematic review has linked smoking to increased risk of tooth loss, periodontal attachment loss, deeper periodontal pockets, and more expansive alveolar bone loss. E-cigarette vapor and conventional cigarettes, especially with flavoring chemicals, have been shown to contribute to the pathogenesis of periodontal disease as well as dysregulated repair responses. The damage done to myofibroblasts can affect the patient’s ability to heal properly due to the decrease in wound contraction (34).

The research explored in this review shows an association between E-cigarette and detrimental effects on the periodontal tissues as well as the inflammatory immune response. However, further studies should be performed to establish a stronger association between vaporized metals and chemicals found in E-cigarette and the effects on the periodontium.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References


Relationship Between Spermatid Specific Thioredoxin-3 Protein and Antisperm Antibody in Patients with Varicocele Compared with a Control Group

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Abstract

The spermatogenesis process are very complex and involves many process to lead to give normal sperm, these sperms a very important to fertility in the men). Protein in the male fertility that is very important which give the significant job in fertilization. The infertile varicocele patient grade 1, 2 and 3 (n= 25 , 25 and 25 respectively) compare with control patient (n=15). The present study Shown spermatid – specific thioredoxin 3 level a significant increase in varicocele patient grade 1,2 and 3 compare with control group (mean± Std. Error 17.97± 0.8 & 22.92±0.75 Respectively ). Also in Antisperm antibody a significant increase in varicocele patient grade 1,2 and 3 compare with control group (mean± Std. Error 73.85±2.71 & 45.6±1.44 Respectively ). Consecutive (r= -0.840, r = -0.745 and r = -0.475). Also theirs a negative correlation between Antisperm antibody level and the sperms concentration, Sperm Progressive motility percent and sperm normal morphology present in varicocele patient grade 1,2 and 3 Consecutive (r= -0.443 , r = -0.370 and r = -0.384 ) .

The result show a positive correlation was found between Spermatid specific thioredoxin-3 protein level and antisperm antibody level in varicocele patients .(The present study conclude that a negative function of Spermatid specific thioredoxin-3 protein and antisperm antibody which that appears on sperm function and sperm parameters , The study showed that spermatid specific thioredoxin-3 protein and antisperm antibody has a harmful effect with the time).

Keywords - Spermatid Specific Thioredoxin-3 Protein (SPTRX3), ASAs, Varicocele

Introduction

The most successful fertility in pets. Also, fertility is a success against sperm DNA damage (1). Thioredoxin family proteins It participates in an antioxidant system and is one of various groups, as it has a role in cellular functions, There are three thioredoxins It is especially expressed in male mammals (2,3). A group of proteins has been discovered in sperm, including Spermatid specific thioredoxin-3 SPTRX3 in the pro-acrosomic granule of round spermatids, which leads to a belief in its role in synthesis (4). There are a number of mechanisms that stimulate autoimmunity and antisperm antibodies including the peripheral acceleration of cells Immuno-type-1, accelerate the secretion of pro-inflammatory cytokines and Tissue necrosis factor (5). Decrease anti-inflammatory cytokines (IL-10, TGF-B) tissue growth factor and/or major (histocompatibility complex, associated molecules for the expression of co-stimulatory molecules expression, and/or the descending complexity of the process of corrosion by immune cells (6). TGF-β is A group of peptides of a special nature, as they control the spread, differentiation and many functions of cells(7). TGF-β acts with TGFA Together, they make a difference as a negative autocrine growth factor , Dysregulation of TGF-β It works to activate and produce signals in apoptosis (8). Many cells synthesize TGF-β There are receptors on all cells that form this peptide , TGF-β1, TGF-β2, and TGF-β3 It has systems and shares the same indicators (9). TGF-β1 It was first discovered in the last century in platelets and its role in recovery (10). Finally it is described as a group
of the safe acids that produce this protein \(^{(11)}\). There are a number of mechanisms that stimulate autoimmune and antisperm antibodies including the peripheral acceleration of cells Immuno-type-1, accelerate the secretion of pro-inflammatory cytokines and Tissue necrosis factor (TNF-a) \(^{(12)}\). Decrease anti-inflammatory cytokines (IL-10, TGF-B) tissue growth factor and/or major histocompatibility complex, associated molecules for the expression of co-stimulatory molecules expression, and/or the descending complexity of the process of corrosion by immune cells \(^{(13,14)}\).

**Materials and Methods**

Samples were collected from semen and serum from infertile patients include (grade 1, 2 and 3) as well as the control group from the infertility center of Al-Sadr Teaching Hospital. As the average age of the patients was \((36.87\pm74)\) years, Years, collected 90 samples and 88 eyes were examined.) A biochemical test was performed on (88) samples had been measured between Spermatid specific thioredoxin-3 protein by immunological method (Enzyme-Linked-Imuno-Sorbent- Assay) by using ELISA reader (Huma Germany origin). Before using the samples, they were placed at room temperature, as well as the reagents. All fluids were used with great care to prevent any errors as the checks were done step by step, All examinations were carried out by the apparatus of the College of Science, Kufa University. Used ELISA kits as follows from the global company (between Spermatid specific thioredoxin-3 protein) (MBS9319574) and ASAs (MBS702581) MYBIOSOURCE USA in Origin).

**Results**

The result show a significant spermatid specific thioredoxin-3 protein level was increase in varicocele patient grade 2 and 3 (mean± Std. Error 17.97± 0.8 & 22.92±0.75 consecutive) also significant ASAs level was increase in varicocele patient grade 2 and 3 (mean± Std. Error 73.85±2.71 & 45.6±1.44 consecutive as in table 1), while the result show negative correlation between(spermatid specific thioredoxin-3 and the sperms concentration); sperm progressive motility percent and sperm normal morphology present in varicocele patient grade 1, 2 and 3 consecutive \((r = -0.443, r = -0.370 \text{ and } r = -0.384 \text{ as in table 3})\).

The result show a positive correlation was found between spermatid specific thioredoxin-3 protein level and antisperm antibody level in varicocele patients).

**Table (1) : The comparison of antisperm antibody concentration in infertile varicocele patient grade 1,2 and 3.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Antisperm Antibodies (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>21.98 ± 0.44</td>
</tr>
<tr>
<td>Grade 2</td>
<td>45.6 ± 1.44</td>
</tr>
<tr>
<td>Grade 3</td>
<td>73.85 ± 2.71</td>
</tr>
<tr>
<td>Control</td>
<td>19.77 ± 0.42</td>
</tr>
</tbody>
</table>

**Table (2) : The comparison of spermatid specific thioredoxin-3 protein with sperm concentration and sperm progressive motile in the serum infertile varicocele patient grade 1, 2 and 3.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Spermatid Specific Thioredoxin-3 Protein (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>9.87 ± 0.12</td>
</tr>
<tr>
<td>Grade 2</td>
<td>17.97 ± 0.8</td>
</tr>
<tr>
<td>Grade 3</td>
<td>22.92 ± 0.75</td>
</tr>
<tr>
<td>Control</td>
<td>9.55 ± 0.41</td>
</tr>
</tbody>
</table>

**Table (3) : The correlation between spermatid specific thioredoxin 3 with sperm concentration and sperm progressive motile in infertile male**

<table>
<thead>
<tr>
<th>Sperm Parameters</th>
<th>Pearson Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration</td>
<td>(r = -0.840)</td>
</tr>
<tr>
<td>Sperm Progressive Motile</td>
<td>(r = -0.745)</td>
</tr>
</tbody>
</table>
Table (4) : The correlation between antisperm antibody with sperm concentration, sperm normal morphology , sperm progressive motile in infertile male

<table>
<thead>
<tr>
<th>Sperm Parameters</th>
<th>Pearson Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration</td>
<td>r = - 0.443</td>
</tr>
<tr>
<td>Sperm Progressive Motile</td>
<td>r = - 0.370</td>
</tr>
<tr>
<td>Sperm Normal Morphology</td>
<td>r = - 0.384</td>
</tr>
</tbody>
</table>

Discussion

The current study showed a significant decrease (p<0.05) of Spermatid specific thioredoxin3 protein level in infertile men grade 1, 2 and grade 3 male infertility compared with control group and show a negative correlation between Spermatid specific thioredoxin3 protein level and sperm concentration , sperm progressive motility in addition to sperm normal morphology these SPTRX3, In biological generation it has an important role to play in the structures of sperm and its dissolution in the semen, They also have many functions In various cellular activities, including DNA synthesis, they also act as antioxidants, regulators of apoptosis h, as well as immune response. These resulted may be effected in these SPTRX3, have a function during the biogenesis of sperm accessory structures but then are degraded within the spermatid cytoplasmic , They also have many functions in Various cellular activities, where it is involved in the defense of sperm, from influences that act as antioxidants that prevent DNA damage and regulate apoptosis and immune response. The protein synthesis is affected by accompanying with increase free radicals (15-17). The study by Moughelinejad et. al., (2018) that showed a negative correlation between Spermatid specific thioredoxin3 protein with sperm parameter when showeda negative correlation between sperms count, sperm progressive motile and sperm normal morphology in Asthenozoospermia and also these study found the elevation of the SPTRX3 level in Asthenozoospermia compare with control.expressed in testis and localized to the Golgi apparatus of mammalian spermatocytes and spermatids. Interestingly, SPTRX-3 is more abundant in defective spermatozoa from infertile male (18).

The current study showed a significant increase (p<0.05) of antisperm antibody level in grade 2, 3 and non-significant increase of grade 1 male infertility compared with control group, while there was significant increase (p<0.05) between grade 1 and grade 2, 3 showmen, and show a negative correlation between antisperm antibody level and sperm concentration , sperm progressive motility in addition to sperm normal morphology , this result may be caused by the disruption of blood-testis barrier and this result is abnormal spermatogenesis (19). Our results agreed with the study that including 238 cases of ASA positive infertility male and 929 ASA negative controls, and show in patient with ASA positive infertility male that have reducing in sperm parameter while the patient with ASA negative controls that have normal sperm parameter and also this study illustrates the effect of ASA on semen liquefaction (20).

Another study that agreement with our result these study including azoospermia (n=22), oligozoospermia (n=9) and control group (n=2), and show in a patient with azoospermia and oligozoospermia elevate of ASA concentration compare with the control group and show the effected of ASA on sperm parameter (21).

Conclusions

Spermatid specific thioredoxin-3 protein and Antisperm Antibody have a negative effective on sperm function when that lead to infertility .

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

Conflict of Interest : Nil

References


Tuberculosiss Arthritis: A Case Report

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Abstract

Tuberculosis Arthritis of the knee joint is rare. A 25-year old female patient presented with pain and swelling of her left knee. The symptoms first appeared two months earlier; she was given glucocorticoid after diagnosed with Rheumatoid Arthritis. The patient did not improve with the treatment. The patient was diagnosed with tuberculosis arthritis according to biopsy and PCR TB result. Biopsy excision of the left knee found granulomatous appearance, which is consistent with tuberculosis, PCR TB examination also found a positive effect. Antituberculosis treatment was started and will be continued for nine months. Tuberculosis arthritis frequently mimics more common etiologies and can be difficult to diagnose. Tuberculosis arthritis may be suspected in a chronic case of joint pain, usually monoarticular, although pulmonary TB may absent.

Keyword: Tuberculosis, arthritis, knee, pain

Introduction

World Health Organization (WHO) declared tuberculosis (TB) as a global emergency since 1993. In 2002, WHO stated that the incidence of TB was 144 in 100,000 people per year with a world population of 6.08 billion people [1]. About 80% of pulmonary TB cases occur in 22 countries, mainly in Southeast Asia, sub-Saharan Africa, and Eastern Europe. In addition to pulmonary TB, there has been a surge in the prevalence of extrapulmonary TB with or without lung involvement in the past 10 years, where 10 to 11% of these extrapulmonary TB cases involve joints and 1 to 3% of these cases involve bones [2,3].

TB arthritis is often presented with several manifestations of subacute or chronic monoarthritis of the weight-bearing joints such as hips, knees, or ankles. This disease has been underdiagnosed for years. The risk factors of TB arthritis are similar to pulmonary TB coupled with the presence of local factors such as pre-existing joint disease including inflammatory arthropathy, crystal arthropathy, joint injury, and surgery. Early diagnosis is required to prevent any delay in the initiation of anti-tuberculosis drugs, thereby reducing morbidity and disability. TB diagnosis requires standard microbiological or histopathological confirmation. Acid-fast bacilli examination of synovial fluid can confirm the diagnosis in only 20-40% cases. Therefore, a synovial biopsy (or other affected tissue) is the gold standard for diagnosing TB arthritis. TB multidrug therapy is not recommended without confirmation. A total of 9 months of treatment for joint and bone TB is supported by some centers [4,5].

Case Description

A 25-year-old woman presented with complaints of worsening pain in the left knee since three months before hospital admission, accompanied by swelling and movement difficulties (Figure 1).

Additionally, in the following two months, the patient also complained of a fluid-filled lump in the chest. The fluid-filled bump was ruptured, producing...
yellowish fluid and pus. The patient denied any respiratory complaint (coughing, hemoptysis, shortness of breath, and chest pain), night sweat, fever, decreased appetite, and weight loss. The patient sought treatment to an internist since the complaint arose and received 8 mg methylprednisolone twice a day, 500 mg calcium carbonate daily, and a capsule of drug combination daily. The patient worked as a salesperson at a souvenir shopping center in Bali. She was a passive smoker. Physical examination revealed swelling and pain during movement on the left knee. Left knee X-Ray showed an impression of joint effusion, soft tissue swelling, and localized osteopenia on the tibial lateral condyle (figure 2).

Chest x-ray examination showed no abnormality. Laboratory tests revealed leukocytosis, increased C-Reactive Protein, and increased erythrocyte sedimentation rate, with negative Rheumatoid Factors (Qualitative RF). Ultrasonography examination found complex joint effusion in the medial compartment of the left knee and left suprapatellar recess with possible synovitis and internal hematoma as well as mild left medial meniscus extrusion. Magnetic Resonance Imaging suggested osteomyelitis in the proximal tibia and distal femur accompanied by Brodie abscess in the medial tibial condyle and septic arthritis. Magnetic Resonance Imaging suggested osteomyelitis in the proximal tibia and distal femur accompanied by Brodie abscess in the medial tibial condyle and septic arthritis. Enhanced chest CT supported mediastinitis appearance on the right superior mediastinum. Excisional biopsy on the left knee showed granulomatous presence, which is consistent with tuberculosis, while synovial fluid was positive on PCR TB. The patient was treated with first line antituberculosis drugs for nine months and followed up clinically.

Figure 1. Patient’s clinical appearance showed swelling and asymmetry on the left knee seen from anterior posterior (A) and lateral (B).

Figure 2. X-Ray (A) anterior posterior and (B) lateral view of the left knee showed an impression of joint effusion, soft tissue swelling, and localized osteopenia on the tibial lateral condyle.
Discussion

Lungs are the central location for TB infection, while skin, digestive tract, tonsil, and placenta are other less common sites for primary TB. Regarding TB arthritis, most infections are due to secondary hematogenous spread via vessels from the primary site. In peripheral diarthrodial TB arthritis, disease originates from adjacent TB osteomyelitis which erodes into the joint space or from direct range to the synovium through the bloodstream resulting in inflammation with edema, muscle spasms, and limited movements. Granulation tissue may cause effusion and pannus formation, which ultimately causes damage to the cartilage. It starts from the edge of the joint eroding into the subchondral cancellous bone, interfering nutrient intake, and loosening the attachment of cartilage to the underlying bone [6]. Severe bone damage can occur following demineralization and necrosis. Cartilage erosion triggers severe muscle spasms with limited joint movement. Imaging in advanced stage shows reduced joint space which is often known as TB arthritis or TB osteoarthritis [4,5,7]. Following this stage, spontaneous para-articular abscesses and external fistula formation occur. The course of tuberculosis synovitis is very typical, and the development of joint damage in TB is much slower than pyogenic infection because mycobacteria do not produce collagenase.

Some conditions have been associated with TB as high-risk factors such as certain socioeconomic classes, immunocompromised hosts, and disturbances in local joints and bone tissue factors. People who use corticosteroid therapy can also be considered as immunocompromised [8]. Other elements of local joints and bone tissue also contribute to the occurrence of TB arthritis. These factors include surgical trauma, intravenous drug usage, and pre-existing joint or bone disease [9]. The patient, in this case, had prolonged use of corticosteroid (methylprednisolone) therapy and osteomyelitis infection (based on MRI) so that the patient became an immunocompromised host, making her more susceptible to TB arthritis. Hips and knees are the most common sites of TB arthritis. Poly/oligoarticular presentation is rare. However, these types should be considered in immunocompromised patients, patients in close contact with TB patients, elder, patients using corticosteroids, or post trauma patients. The early presentations of TB joint are swelling (20%), stiffness (10%), and weakness or limitation of movement. The bones are usually warm and sometimes characterized by thigh muscle atrophy. Synovial hypertrophy and effusion occur in most patients. Muscle cramps and synovial effusion can cause flexion deformity. As the disease progresses, articular cartilage damage occurs coupled with decreased joint space that can lead to its narrowing and irregularity of the cartilage surface. Worsening left knee pain along with swelling and movement difficulties in this patient mimic the symptoms of rheumatoid arthritis, leading to corticosteroid therapy.

Histopathology and microbiology technique remain the gold standards for the diagnosis of TB arthritis and osteomyelitis. Tissue biopsy of representative lesions is the fastest method, one of which is synovial biopsy with more than 90% accuracy. In contrary, synovial fluid smear for TB is positive in only 20-40% cases, while culture can produce positive results in up to 80% cases. Analysis of synovial fluid shows unspecific findings of inflammation, even deficient glucose levels support the diagnosis [10]. Typical radiographic images of TB arthritis are called Phemister triads, characterized by juxta-articular osteoporosis, osseous erosion located at the edges, and gradual narrowing of the joint space. This is different from rheumatoid arthritis and pyogenic arthritis, where joint space narrowing occurs at the beginning of the disease.

The multidrug administration is mandatory because TB bacilli quickly develop into drug-resistant bacilli with different growth patterns between TB bacilli in the same TB location. It is complicated for the TB bacilli to become resistant if two or more drugs are used in combination. The challenge of initial drug resistance can be ignored in TB arthritis due to its small number of microorganisms (10^5-10^6). The optimal duration of therapy for extrapulmonary TB, including TB arthritis, remains unclear. The latest recommendation is nine months of treatment to prevent disability. Second-line drugs are only used for the treatment of TB that is resistant to first-line drugs. TB arthritis treatment outcome is evaluated only by the clinical method. This patient was given first-line drug treatment in the form of 3 fixed-drug combinations (FDC) daily for two months in the intensive phase according to the patient’s body weight and was planned to be continued with an
awkward stage for seven months (total of at least nine months). This regimen was by recommendations for joints and bones TB.

Occasionally, surgical intervention is needed with clear indications, such as diagnostic interventions (synovial biopsy or lesions), inadequate response to drug therapy, adequate joint lavage or arthroscopic drainage, bone debridement, aspiration or even drainage (in case of abscess) [6]. As a common rule, multidrug therapy is required for four weeks to stabilize the general condition of the patient before the surgery is performed. At the beginning of the treatment, immobilization can be helpful to eliminate pain and prevent any deformities.

Conclusions

TB arthritis can occur primarily as subacute or chronic monoarthritis of the weight-bearing joints such as hips, knees, or ankles. Back pain with ‘spooky’ clinical features is another general presentation. The diagnosis of TB arthritis requires standard microbiological or histopathological confirmation. Synovial biopsy (or other affected tissue) remains as the gold standard for TB arthritis diagnosis. TB multidrug treatment is not recommended without a confirmed TB diagnosis.

Conflict of Interest: The authors report no conflict of interest.

Acknowledge: We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

Funding: None

Ethics Statement

All procedures performed in studies/case report were in accordance with the ethical standards of the Ethics Committee in Faculty of Medicine, Udayana University, Bali, Indonesia. The authors explains the aimed, benefits, and rights of the participant during the process of collecting data to the patient’s guardian, if the participant agrees we ask the participant to fill out an informed consent sheet.

References

Peritoneal Tuberculosis as a Final Result of Diagnostic Conundrum: A Case Report

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Abstract
Peritoneal tuberculosis is a disease which can mimic malignancy especially in women present with ascites and elevated CA125 levels. It always should be considered in the differential diagnosis. Peritoneal tuberculosis with ambiguous patient symptoms and diagnostic difficulties still poses a great challenge in clinical practice. A 33 years old woman was admitted to hospital with complaints of abdominal pain and shortness of breath. Initially, elevated serum antigen CA-125, ascites, pleural effusion, and multiple suspicious nodules in the lungs and abdominal cavity on CT were found, thus, diagnosing advance stage ovarian cancer. Biopsy laparotomy was performed, and post-operative histological examination was peritoneal tuberculosis. The patient was treated with first-line antituberculosis agents and discharged home. Almost in all other cases, peritoneal tuberculosis is diagnosed only after surgical intervention.

Keywords: ascites, pleural effusion, ovarian cancer, peritoneal tuberculosis, CA125

Introduction
Diagnosis of extrapulmonary tuberculosis is difficult. Tuberculous peritonitis, accounting for 1%-2% of all tuberculosis cases, is caused by abdominal or pelvic tuberculosis that involves the peritoneum (1). The postulated mechanism is tubercular bacilli reaching the peritoneal cavity via the bloodstream or by direct spread from the contiguous infected small intestine, lymph nodes, and fallopian tubes (2). Patients usually have nonspecific symptoms and signs, such as abdominal pain, abdominal distension, and poor appetite. The serum cancer antigen (CA)-125 can be elevated in both ovarian cancer and peritoneal tuberculosis. Similar clinical and image findings lead to diagnostic difficulty and the challenge of distinguishing these two disease entities.

Case Report
A 33-year-old nulliparous woman was admitted to hospital with a two-month history of tenderness in the abdomen who referred from another center to us with reported ascites in ultrasonography and elevated CA-125. She had fevers, chills, or night sweats and weight loss. The significant patients past medical history were only abortion five months ago. Her positive physical findings were limited to her abdomen, which was grossly distended, and although no masses were palpable, ascites was present. On pelvic examination, the cervix and vaginal walls were without gross lesions, and the adnexa and uterus were not palpable secondary to the ascites. Primary ovarian malignancy was suspected, and the patient was referred to the gyne-oncology clinic.
Laboratory showed a normal white cell count, a haemoglobin of 12.02 g/dL and elevated CA-125 to 460.78 U/mL (normal range, <35 U/mL), CEA level 0.39 (normal range: 0–3.0 ng/mL), B-HCG level < 2 (normal range 0-5). Abdominal sonography revealed ascites, and no mass were found. Abdominopelvic CT revealed ascites, subdiaphragmatic nodules, enlarged parailiaca lymph nodes, and adnexa mass (figure 1). The liver, spleen, and kidneys appeared within normal limits. A few days later she developed left pleural effusion, and a chest tube was inserted (figure 2). Pleural fluid was negative for AFB and malignancy. Chest CT showed multiple subcentimeter nodules as well as a ‘tree in bud’ appearance throughout the lung parenchyma. Sputum analysis did not reveal any mycobacteria. The ascitic volume decreased but became denser. The patient then agreed to diagnostic laparoscopy. At laparoscopy, the peritoneal cavity was difficult to enter with thick adhesions and miliary seedlings (figure 3).

No intraabdominal mass was seen. Frozen section facilities were not available; however, biopsies were taken for tissue diagnosis, leaving the uterus and ovaries intact. Histology revealed caseating granulomas with epithelioid and Langhan’s type of giant cells (figure 4). The final diagnosis from the biopsy was peritoneal tuberculosis. Symptoms resolved, and the CA125 levels normalized after two months of antituberculosis therapy. She is to continue her treatment for a total of 6 months.

Figure 1. CT Abdominopelvic showed ascites, subdiaphragmatic nodules, enlarged parailiaca lymph nodes and adnexa mass at anterior posterior (A); lateral (B).
Figure 2. Chest CT showed pleural effusion and multiple subcentimeter nodules as well as a ‘tree in bud’ appearance throughout the lung parenchyma (A & B).

Figure 3. Intraoperative findings Miliary seedlings on peritoneum and serosal surface of bowel with dense adhesions.
Figure 4. Histopathology showed granulomatous inflammation - caseating granulomas with epithelioid and Langhan’s type giant cells.

Discussion

The diagnosis of any extrapulmonary forms of TB is especially difficult, particularly for the peritoneal type as the symptoms and physical findings of the disease do not substantiate the diagnosis. Diagnostic delays and delayed initiation of treatment can lead to high morbidity and mortality rates (3). The signs and symptoms typically associated with advanced ovarian carcinoma include abdominal distension, ascites, and pelvic or adnexal masses (4). Many of these women go on to have radical surgery due to the difficulty of definitive preoperative diagnosis of ovarian cancer and the low negative predictive value of ascitic fluid cytology (5).

Abdominal tuberculosis can present with a similar clinical scenario, with most cases diagnosed incidentally at laparotomy. This has often led to unnecessary extensive surgery, frequently in women of reproductive age (5, 6). CA125 lacks specificity, elevated in many conditions, including tuberculosis (7). One study showed that CA125 titers higher than 1,000 U/ml correlated with malignancy (8). However there was a reported case of peritoneal tuberculosis with a CA125 level of 1,081 U/ml (9). This means that absolute CA125 levels are not definitive in determining malignant versus nonmalignant causes. Although CA125 levels are useful in monitoring response to therapy in ovarian carcinoma, there has been no report of CA125 levels declining without treatment in malignant conditions (10). Chest radiographs can be normal in patients with peritoneal tuberculosis, approximately 40% of the time (11).

Abdominal CT in tuberculous peritonitis typically shows smooth, strongly enhancing peritoneal thickening and a dirty omentum. Peritoneal carcinomatosis, however, commonly shows nodular peritoneal thickening and a nodular or caked omentum. Other findings that suggest a diagnosis of tuberculosis include dense ascites, caseous nodes and soft-tissue mesenteric and omental infiltration (12). There are high false negative rates for tuberculosis skin tests and AFB detection in pleural and peritoneal fluid (13). For preoperative detection of tuberculosis, ascitic fluid adenosine deaminase (ADA) and PCR
analyses have proven to be useful. However, these tests may not be available in all settings (14).

The determination of ADA in peritoneal fluid is also helpful for TB diagnosis. This enzyme involves the conversion of adenosine to inosine in the catabolism of purines, and its levels are elevated in peritoneal TB due to stimulation of T-lymphocytes by the immune response to antigens of mycobacterial cells. The estimation of ADA levels in ascitic fluid have 98% specificity, 96% sensitivity, 88% negative predictive value, and a high positive predictive value of 95% (15). Levels more than 40 units per liter are indicative of tuberculosis (16). Ultrasound-guided trucut biopsy has also been shown to be a valuable first-line approach. All investigations for diagnosing peritoneal tuberculosis have proven to be inconclusive only histopathologic investigation is confirmatory. Diagnostic laparoscopy or laparotomy is usually necessary however, for definitive diagnosis, where intraoperative frozen sections can aid in avoiding unnecessary extensive surgery.

**Conclusion**

By the information given in this case report, one can understand that peritoneal tuberculosis can often mimic advanced ovarian cancer and peritoneal carcinomatosis. It should always be considered in the differential diagnosis, but the diagnosis is rarely easy for clinicians. True diagnosis and then correct and careful follow-up can save the patient’s life, and doctors should start the treatment as soon as possible.

**Acknowledgments:** We thank the departments of gynecology, radiology, and pathology at the Arifin Achmad General Hospital for their assistance in managing this patient. We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

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**Conflict of Interest:** The authors report no conflict of interest in this publish.

**Funding:** one

**Reference**


The Relation between Exercise Duration and Intensity on Phosphocreatine (PCr) Level: an Article Review

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Abstract

Exercise is a physical activity that planned, structured, and sustainable. Exercise has 4 criteria, that is frequency, intensity, type, and duration. During exercise, phosphocreatine (PCr) depletion increases, and early intracellular acidosis has occurred. These changes contribute to a decrease in training capacity in terms of training duration. This literature review aimed to determine the relationship between the intensity and duration of exercise with the concentration of PCr and recovery factors. The literature study has carried out by selecting an Experiment research design. The results of the review showed that PCr was the largest energy contributor in the first 10 seconds of exercise in the heavy-intensity exercise where phosphocreatine triggers energy without oxygen or anaerobic. The recovery of PCr influenced by a person’s health condition and age. The concentration of PCr in children was higher than in adults because of the relatively high rate of oxidative ATP formation. It proved that there was a significant relationship between PCr and the duration and intensity of exercise.

Keywords: Exercise duration, Exercise intensity, PCr, Phosphocreatine

Introduction

Health is considerable for humans. Everyone must be healthy to maintain physical fitness so that they can carry out daily activities. According to the Indonesian Law No. 23 of 1992 concerning health states that health is a state of well-being of body, soul, and society which enables everyone to live productively socially and economically(1). Health can be pursued in various ways, one of which is by exercising.

Exercise is a physical activity that has planned, structured, and continuous by involving regular and repeated body movements to improve physical fitness and achievement(2). Exercise is useful for maintaining and increasing mobility and independence to move in human bio-psycho-sociologic life(3). Exercise has four criteria, that is frequency, intensity, type, and duration(4).

Exercise can stimulate the disruption of homeostasis and change the physical and chemical environment of cells. Exercise can cause body temperature to increase, increase blood acidity, decrease oxygen in fluids, and increase CO2. Environmental changes in the body start at the receptors, namely body cells that will stimulate complex response pathways. This pathway causes changes in nerve activity (nerve pathways), hormonal changes (hormonal pathways), and exchanges in specific pathways (intrinsic pathways). Also, chemical, mechanical and thermal stimuli affect changes in metabolic, cardiovascular, and ventilator functions to meet increased demand(5).

Exercises of longer duration (2-3 minutes) that rely primarily on oxidative metabolisms, such as swimming and long-distance running, are classified as aerobic activities. Many sports activities require a combination of anaerobic and aerobic metabolism. In stop and go sports, about 60% -70% of energy comes from ATP storage from phosphocreatine (PCr) and anaerobic glycolysis, the remaining 30% from oxidative processes(6).

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Phosphocreatine (PCr) is a high-energy compound that has a high-energy phosphate bond that can be hydrolyzed into energy and can resist ATP. In physical activity or strenuous exercise such as sprinting, PCr in the skeletal muscles makes a big contribution for the first 10 seconds. The storage of PCr will quickly run out but, in the first few seconds of exercise, PCr provides a significant buffer before other aspects of metabolism are activated\(^7\).

During exercise, physical activity, or exercise, PCr depletion increases, and early intracellular acidosis has occurred. These changes contribute to a decrease in training capacity related to exercise duration\(^8\).

**Material and Method**

This study used a literature review method. The article search strategy has carried out by using international or national journal articles that were searched through Google Scholar and PubMed. Through the keywords searched were exercise duration, exercise intensity, phosphocreatine, and PCr, from Google Scholar website founded 31 articles of search results and on the PubMed website as many as 13 articles. Then, screening has carried out by selecting articles that were relevant to the topic, namely the effect of exercise duration on phosphocreatine (PCr) levels. The articles selected were articles with inclusion criteria using Experiment research methods. The exclusion criteria in this literature review were articles that were not related to the topic of the effect of exercise duration and intensity on phosphocreatine levels.

**Results and Discussion**

Various types of sports can be an option to maintain body fitness. However, it is considerable to note in planning exercise activities that at least four criteria are met, namely the frequency of exercise, intensity/weight of exercise, type of sports activity, and length of time exercising\(^4\). In sports, various kinds of metabolism will produce different types of waste products, one of which is creatinine.

Creatinine is a chemical waste molecule that results from muscle metabolism. Creatinine has produced from creatine, a molecule that is essential for energy production in muscles. About 2% of keratin in the body is converted to creatinine every day. Creatinine
is transported through the bloodstream to the kidneys. Creatinine levels are determined by the amount of muscle mass (protein catabolism rate), in addition to how our body’s metabolic activity, for example, increases when we are sick (heat/infection). Creatinine is produced during skeletal muscle contraction through the breakdown of creatinine phosphate\(^9\).

Muscles use phosphocreatine during the first few seconds of intense muscle contraction, such as during weight lifting or sprinting. Unlike aerobic contraction, which utilizes oxygen to produce energy, phosphocreatine triggers energy without oxygen or is anaerobic\(^10\).

Bogdanis et al. stated that aerobic metabolism provides a significant part (~ 49%) of energy during the second sprint, whereas PCr availability is important for high power output during the initial 10 s\(^11\). This is supported by the statement of Hall and Trojan that creatine monohydrate can improve muscle performance in a short duration, and high-intensity resistance training will rely on the transport of phosphocreatine to become adenosine triphosphate so that an increase in the level of total creatine in cells will allow for the faster synthesis of phosphocreatine\(^10\). Increasing the creatine level in the body can delay fatigue because creatine can be re-synthesized and sent back to the site of ATP use more quickly\(^12\). Fatigue during short-term high-intensity exercise is related to the availability of PCr because PCr can regenerate ATP at very high rates, and its concentration in muscle is limited\(^13\).

The energy at the start of a workout or exercise that uses very high-intensity muscle work (85% -100% of maximum capacity) and has a short duration (up to 10 seconds) has determined from the small amounts of ATP and PCr stored in muscle cells \(^5,11\). The total energy available in the stored ATP-PCr is sufficient for short duration exercises, such as lifting weights, high jumps, or 10-second sprints\(^14\).

The amount of energy generated from the PCr has limited because of the intramuscular pathway. The high anaerobic demands on the muscles can decrease muscle PCr concentration. At the same time, in a short period of maximum exercise, the anaerobic utilization of muscle PCr and glycogen will trigger muscle contraction\(^15\). Short-term contractions are associated with metabolic changes in the muscle so as decreased muscle phosphocreatine\(^16\).

According to Haseler, Hogan, and Richardson, in skeletal muscle, PCr recovery from submaximal exercise is a measure of muscle-oxidative capacity. PCr recovery was significantly altered by FIO2 and after submaximal exercise, in normoxic conditions, PCr recovery was limited by O2 availability\(^17\). Also, The PCr recovery time constant is prolonged in patients with the symptomatic peripheral arterial disease (PAD), whether differences in PCr recovery time result entirely from changes in tissue blood flow, alterations in skeletal muscle at a cellular level, or a combination of both deserves further investigation\(^18\).

In patients with PAD symptoms, the PCr recovery time constant is longer than that of ordinary people, so it is recommended for PAD sufferers to do sports with a long duration of intensity\(^19\). Also, there is the notion that phosphate regulation and muscle O2 utilization is fully mature in peri-pubertal children, which may be due to the comparable capacity for mitochondrial oxidative phosphorylation in child and adult muscles\(^19\). The relatively higher rate of oxidative ATP build-up in children’s muscles to mask the ATP demand from high-intensity intermittent exercise compared to adults, allows children to start each exercise interval with a much higher concentration of PCr and lead to more muscle acidification. Low overall\(^20\).

**Conclusion**

From the review above, it can conclude that PCr has a significant relationship with exercise duration and intensity. PCr is the largest energy contributor in the first 10 seconds of exercise in a heavy-intensity exercise where phosphocreatine triggers energy without oxygen or anaerobic, so with an increase in creatine phosphate capacity, very high-intensity exercise performance can be improved. PCr concentration is not only influenced by the duration and intensity of exercise, but also by health conditions and age.

**Acknowledgment**

We thank all the panelists involved in this study. The researchers also extend the gratitude to Direktorat Riset dan Pengabdian Masyarakat, Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset.
Conflict of Interest: No conflict of interest real or perceived.

Source of Funding: This article was funded by Direktorat Riset dan Pengabdian Masyarakat, Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset dan Teknologi/ Badan Riset dan Inovasi Nasional through Program Disertasi Doktor (PDD) grant 2020.

Ethical Clearance: The ethical clearance was obtained from The Committee of Ethical Approval in the Faculty of Nursing Universitas Airlangga No. 1974-KEPK.

References
The Effect of Mixed Liquor Administration on The Johnsen’s Score and The Number of Sertoli Cells and Leydig Cells on The Wistar Strain White Rats (*Rattus norvegicus*)

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Abstract

To analyze the effect of mixed liquor administration on the Johnsen’s score and the number of Sertoli cells and Leydig cells on the Wistar strain white rats (*Rattus norvegicus*). A total of 28 rats were divided into 4 groups: C, P1, P2, and P3. Rats were given mixed liquor with different dose, namely P1 (1 ml), P2 (2 ml), and P3 (4 ml) each day for 14 days, then compared to C group which was given 4 ml of distilled water using an oral gavage. Mixed liquor consists of 20% ethanol and 4% methanol. The histopathological features were evaluated by the Johnsen’s score, the number of Sertoli cells, and Leydig cells in cross-sectional preparation of rat testicular tissue with 400× magnification. Data were analyzed using the Kruskal-Wallis test and One-Way ANOVA test with a confidence level of \( p < 0.05 \). The P3 group had the lowest Johnsen’s score and the number of Sertoli cells, 6.442±0.293 and 5.942±0.674, respectively. A significant decrease in increased dose occurred in the Sertoli cell count but not in the Johnsen’s score. Group P2 had the lowest number of Leydig cells, 6.421±0.360. The administration of mixed liquor caused a decrease in Johnsen’s score and the number of Sertoli cells and Leydig cells on the Wistar strain white rat (*Rattus norvegicus*).

Keywords: mixed liquor, spermatogenic cells, Sertoli cells, Leydig cells.

Introduction

Alcoholic drinks or liquor are drinks that contain ethyl alcohol or ethanol (C\(_2\)H\(_5\)OH) which are known to cause addiction.[1] Addiction that is satisfied continuously will cause a tolerance effect, that is a desire to increase the dose in order to get the same effect. [1,2] This type of mixed alcoholic drink is often called mixed liquor or *miras oplosan* in Indonesian. Ethanol, which is commonly consumed has negative effect on the male reproductive organs and is associated with several incidence of infertility. [3,4] A study by Fauziah proves that *arak bali*, Balinese wine can reduce the number of spermatogenic cells and the size of the seminiferous tubules of mice. [5] In another study by Antari, *arak bali* also had an impact on lowering the quality of spermatozoa and testosterone levels. [6]

Methanol is one of the substances that is often added because it is effective to increase the effect of drunk. [7] Mixed liquor containing methanol is also reported to cause some death in several areas in Indonesia.[8,9] Ethanol is known to have an effect on male infertility, so the addition of methanol to mixed liquor may have more impact on male reproductive organs. [3,4,10]

Apart from the substance themselves, cell damage is also caused by metabolites of these substances. [11-14] The transformation of ethanol into acetaldehyde and free radicals is known to occur directly in the testes. [15,16] Cell damage by ethanol and methanol occurs due to increased levels of ROS, decreased GSH levels, and
decreased ATP synthesis.\textsuperscript{11-13} Therefore, this study aims to analyze the effect of mixed liquor administration on the Johnsen’s score and the number of Sertoli cells and Leydig cells on the Wistar strain white rats (\textit{Rattus norvegicus}).

\section*{Materials and Methods}

This study is an experimental laboratory study with post-test only control group design. This study used 28 male Wistar rats (\textit{Rattus norvegicus}) which were divided into four groups. The treatment given was administration of mixed liquor with an oral gavage for 14 days to determine its effect on the histopathological features of rats’ testes.

Mixed liquor was given at different doses in each group, namely 1 ml (P1), 2 ml (P2), and 4 ml (P3). The composition of mixed liquor was 20\% ethanol and 4\% methanol. The composition was determined based on the results of gas chromatography tests of mixed liquor samples.\textsuperscript{17} This study used 28 rats in each group, so the total volume of mixed liquor to be prepared for 14 days was $14 \times [7 (1 + 2 + 4)] = 686$ ml. 1000 ml of mixed liquor was made to simplify calculations and anticipate spills. The mixed liquor was made by mixing 208.3 ml of 96\% ethanol, 40.81 ml of 98\% methanol, and 750.89 ml of distilled water. Assessment of the histopathological features was carried out by identifying spermatogenic cells, Sertoli cells, and Leydig cells referring to Pintus et al.\textsuperscript{18} The data was analyzed by statistical software product and service solution 20 for Windows (SPSS 20).

\section*{Results and Discussion}

The samples of this study were 28 adult male rats with 7 rats in each group. Observation of spermatogenic cells, Sertoli cells, and Leydig cells were made on both testes, so that each rat produced two data which were then averaged. The analysis was performed on 28 datas. The data of Johnnsen’s score was an ordinal data, so non-parametric test (Kruskal-Wallis) was chosen. The result showed that the data of Johnnsen’s score had significant difference ($p = 0.000$). Therefore, the analysis was continued with Mann-Whitney test.

\begin{table}[h]
\centering
\caption{Comparison of the Johnsen’s score in each group.}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Group} & \textbf{N} & \textbf{Mean±SD} & \textbf{p-Value} \\
\hline
C & 7 & 8.350±0.165 & 0.000* \\
\hline
P1 & 7 & 7.550±0.104 & \\
\hline
P2 & 7 & 7.478±0.152 & \\
\hline
P3 & 7 & 6.442±0.293 & \\
\hline
\end{tabular}
\end{table}

\* $p <0.05$, significantly different by statistic

\begin{table}[h]
\centering
\caption{Mann-Whitney test analysis for the comparison of the Johnsen’s score and the number of Leydig cells.}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Comparison between} & \textbf{C vs P1} & \textbf{C vs P2} & \textbf{C vs P3} & \textbf{P1 vs P2} & \textbf{P1 vs P3} & \textbf{P2 vs P3} \\
\textbf{Groups} & & & & & & \\
\hline
\textbf{p-value of Johnsen’s} & 0.002* & 0.002* & 0.002* & 0.301 & 0.002* & 0.002* \\
\textbf{score} & & & & & & \\
\hline
\textbf{p-value of the number of} & 0.002* & 0.002* & 0.002* & 0.083 & 0.442 & 0.025* \\
\textbf{Leydig cells} & & & & & & \\
\hline
\end{tabular}
\end{table}

\* $p <0.05$, significantly different by statistic
Table 3. Comparison of the number of Sertoli cells in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Normality</th>
<th>p-Value</th>
<th>Homogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7</td>
<td>11.542 ± 0.401</td>
<td>0.380</td>
<td>0.000*</td>
<td>0.291</td>
</tr>
<tr>
<td>P1</td>
<td>7</td>
<td>9.657 ± 0.504</td>
<td>0.552</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>7</td>
<td>7.557 ± 0.53</td>
<td>0.793</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>7</td>
<td>5.942 ± 0.674</td>
<td>0.361</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Post-hoc analysis for the comparison of the number of Sertoli cells.

<table>
<thead>
<tr>
<th>Comparison Between Groups</th>
<th>Mean Difference</th>
<th>CI95%</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
</tr>
<tr>
<td>C vs P1</td>
<td>1.885</td>
<td>1.094</td>
<td>2.677</td>
</tr>
<tr>
<td>C vs P2</td>
<td>3.985</td>
<td>3.194</td>
<td>4.777</td>
</tr>
<tr>
<td>C vs P3</td>
<td>5.600</td>
<td>4.808</td>
<td>6.391</td>
</tr>
<tr>
<td>P1 vs P2</td>
<td>2.100</td>
<td>1.308</td>
<td>2.891</td>
</tr>
<tr>
<td>P1 vs P3</td>
<td>3.714</td>
<td>2.922</td>
<td>4.505</td>
</tr>
<tr>
<td>P2 vs P3</td>
<td>1.614</td>
<td>0.822</td>
<td>2.405</td>
</tr>
</tbody>
</table>

*p <0.05, significantly different by statistic

Table 5. Comparison of the number of Leydig cells in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Normality</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7</td>
<td>11.378 ± 0.297</td>
<td>0.006</td>
<td>0.000*</td>
</tr>
<tr>
<td>P1</td>
<td>7</td>
<td>6.907 ± 0.511</td>
<td>0.806</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>7</td>
<td>6.421 ± 0.360</td>
<td>0.608</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>7</td>
<td>7.150 ± 0.621</td>
<td>0.304</td>
<td></td>
</tr>
</tbody>
</table>

*p <0.05, significantly different by statistic

The Mann-Whitney test showed that the Johnsen’s score of the control group (C) was significantly higher than
the group receiving mixed liquor with different doses: 1 ml (P1), 2 ml (P2), and 4 ml (P3) with the value of $p=0.002$ on each compared group. So, all of the treatment groups (P1, P2, and P3) had significant reduction in Johnsen’s score compared to the control group. Besides, the significant reduction was also found in the increasing dose from P1 group to P3 group (7.550 vs 6.442; 0.002); and from P2 group to P3 group (7.478 vs 6.442; 0.002), but there was no significant reduction from the P1 group to P2 group (7.550 vs 7.478; 0.301).

The data normality of the number of Sertoli cells was tested by Shapiro-Wilk test; the result showed that each group was normally distributed ($p>0.05$). The analysis was continued by the One-Way ANOVA test and showed a significant difference in the number of Sertoli cells ($p=0.000$). The homogeneity test was also conducted to determine the selection of the post-hoc test method; the result showed that the data was homogeneous. Therefore, the analysis was continued with the post-hoc test.

The post-hoc test showed that the number of Sertoli cells of the control group (C) was significantly higher than the group receiving mixed liquor with different doses with the value of $p=0.000$ on each compared group. So, all of the treatment groups (P1, P2, and P3) had significant reduction in the number of Sertoli cells compared to the control group (C). Besides, the significant reduction was also found in the increasing dose from P1 group to P2 group (9.657 vs 7.557; 0.000); from P1 group to P3 group (9.657 vs 5.942; 0.000); and from P2 group to P3 group (7.557 vs 5.942; 0.000).

The data normality of the number of Leydig cells was tested by Shapiro-Wilk test; the result showed that the control group (C) was not normally distributed ($p=0.006$) and the remaining groups were normally distributed ($p>0.05$). The analysis was continued by the non-parametric test (Kruskal-Wallis) and showed a significant difference in the number of Leydig cells ($p<0.05$). Therefore, the analysis was continued with the Mann-Whitney test.

The Mann-Whitney test showed that the number of Leydig cells of the control group (C) was significantly higher than the group receiving mixed liquor with different doses with the value of $p=0.002$ on each compared group. So, all of the treatment groups (P1, P2, and P3) had significant reduction in the number of Leydig cells compared to the control group (C). But, there were no significant difference in the increasing dose between P1 group and P2 group (6.907 vs 6.421; 0.083); and between P1 group and P3 group (6.907 vs 7.150; 0.442). There was significant difference between P2 group and P3 group (6.421 vs 7.150; 0.025) but P3 group was higher than P2 group and also P1 group.
The study showed that there was a significant decrease of the Johnsen’s score in the treatment group (P1, P2, and P3) compared to the control group (C) \((p < 0.05)\). It means that mixed liquor administration can reduce spermatogenesis activity and the number of spermatogenic cells, as evidenced by a decrease in the Johnsen’s score at the increasing doses.\(^{[19]}\) Increasing dose of mixed liquor can increase the testicular damage of rats.\(^{[20]}\) This study found that there was no significant decrease in the Johnsen’s score from the P1 to P2 group. In addition, the spermatogenic cells were still not protected by antioxidants from the toxic effects of ethanol.\(^{[21]}\)

The decrease in the number of Sertoli cells per tubule in the increasing of dose was found to be significant \((p < 0.05)\). These result is in line with a study by Figueiro et al. that there was a significant decrease in the number of Sertoli cells in all treatment groups of mice that were given ethanol.\(^{[22]}\) The Sertoli cells are part of the testicular blood barrier (BTB), which separate the basal compartment and the adluminal compartment.\(^{[23]}\) BTB is a collection of protein structures consisting of several types of cellular junctions.\(^{[23]}\) The testes are organs that are susceptible to alcohol because alcohol can penetrate the BTB.\(^{[24]}\) This can occur because the structural proteins that make up the tight junction are interfered due to alcohol.\(^{[25]}\)

This study found a significant decrease in the number of Leydig cells on the treatment group (P1, P2, and P3) against the control group (C) \((p < 0.05)\), while there was no significant decrease \((p > 0.05)\) between treatment groups (P1, P2, and P3) as the dose increased. Several studies showed that there was a decrease in testosterone levels in the ethanol-treated group.\(^{[20,21]}\) A decrease in testosterone levels was followed by an increase in LH levels after ethanol administration for 4 weeks as a compensatory feedback.\(^{[21,26]}\) In this study, there was a decrease in the number of Leydig cells from P1 to P2 group. Moreover, the P3 group had a higher number of Leydig cells than the P1 group. It might be due to increase in LH level that stimulates Leydig cell progenitor proliferation.\(^{[27]}\)
Conclusion

The administration of mixed liquor caused a decrease in Johnsen’s score and the number of Sertoli cells and Leydig cells of the Wistar strain white rat (Rattus norvegicus).

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: This study supported by the Ministry of Education and Culture of the Republic of Indonesia.

Acknowledgements: The author would like to express the gratitude to all lecturers during education period in Faculty of Medicine, Universitas Airlangga. We thank to Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Biological Activity Investigation of Phytocomponents in Mangosteen (Garcinia mangostana L.): In Silico Study

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Abstract
Indonesia has abundant medicinal plants, which have been historically used by the population in treating diseases for generations. Traditional Indonesian medicine and the medicinal plants used could lead to the discovery of novel drugs. The mangosteen or Garcinia mangostana L. is a well-known medicinal plant that has been used to treat various diseases worldwide. The pharmacological activities and phytochemical composition of the whole plant of mangosteen have been investigated and identified by scientists in recent decades. Therefore, this study aims to generate the prediction of the biological activity of phytocomponents in mangosteen. In this study, we extracted 18 phytocomponents of mangosteen from PubChem, an open chemistry database at the National Institutes of Health (NIH), USA. Then, we predicted the pharmacokinetic properties and druglike nature of the phytocomponents using the SwissADME web server, Swiss Institute of Bioinformatics, Swiss. Furthermore, PASS (Prediction of Activity Spectra for Substances) web resource has been employed as a strong potential tool to predict the biological activity. In summary, we revealed the biological activity of 18 phytocomponents of the mangosteen. However, further trials, such as in vitro and in vivo evaluation, are needed to prove the validity of these findings.

Keywords: Biological activity, Garcinia mangostana L., in silico.

Introduction
Indonesia has rich sources of natural medicines and traditional medicine preparations that have been used by most of the population for generations[1,2,3]. A direct advantage of traditional medicine formulations to the community is the ease of obtaining them[4]. The demand for plants used as traditional medicine formulations by the community is also increasing because plant-derived medicines have proven to be healthier and do not cause as many side effects as those derived from chemicals. However, a problem with traditional medicine formulations is the lack of adequate knowledge and information about the various types of plants commonly used as ingredients and their methods of use[5,6].

Garcinia mangostana L. or mangosteen appertain to the family of Clusiaceae and genus Garcinia[7]. Garcinia is a large genus that consists of around 400 species originated from Malay Peninsula, East India, and Southeast Asia, including Indonesia. Moreover, based on the morphological and cytological studies, it can be suggested that the mangosteen is originated from Southeast Asia[8]. As a matter of fact, mangosteen is a plant as traditional medicine for hundreds of years worldwide[9].

Furthermore, mangosteen contains bioactive compounds such as xanthones, terpenes, anthocyanins, tannins, phenols, and some vitamins[10,11]. In fact, mangosteen’s pericarp has many important benefits for health[12,13,14]. The main compounds in the content of mangosteen’s pericarp are xanthones[15], such as alpha-mangostin[16], gamma-mangostin[3,17], 8-deoxygartanin, garcinone E, mangostanol[18], beta-mangostin[19], tovophyllin A and B[20],

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DOI Number: 10.37506/ijfmt.v15i1.13522
mangostenin\textsuperscript{[21]}, and mangostenones C, D, and E\textsuperscript{[22]}. The main xanthone derivative is alpha-mangostin, this compound has a variety of pharmacological activities such as antidiabetic\textsuperscript{[23,24]}, antioxidants, and anti-inflammatory\textsuperscript{[12,13,14,25]}. Therefore, this study aims to generate the prediction of the biological activity of phytocomponents in mangosteen.

**Materials and Methods**

**Data retrieval**

We extracted 18 phytocomponents of mangosteen from PubChem, an open chemistry database at the National Institutes of Health (NIH), USA (Table 1). We revealed the Canonical SMILES of the 18 phytocomponents and submitted them to the SwissADME web server for further analysis.

**Table 1. Phytocomponents of the mangosteen revealed from the PubChem database.**

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Formula</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alpha-mangostin</td>
<td>(C_{24}H_{26}O_6)</td>
<td>410.46 g/mol</td>
</tr>
<tr>
<td>2</td>
<td>Beta-mangostin</td>
<td>(C_{25}H_{28}O_6)</td>
<td>424.49 g/mol</td>
</tr>
<tr>
<td>3</td>
<td>Gamma-mangostin</td>
<td>(C_{25}H_{30}O_6)</td>
<td>396.43 g/mol</td>
</tr>
<tr>
<td>4</td>
<td>Gartanin</td>
<td>(C_{22}H_{24}O_6)</td>
<td>396.43 g/mol</td>
</tr>
<tr>
<td>5</td>
<td>Garcinone A</td>
<td>(C_{23}H_{24}O_5)</td>
<td>380.43 g/mol</td>
</tr>
<tr>
<td>6</td>
<td>Garcinone B</td>
<td>(C_{23}H_{22}O_6)</td>
<td>394.42 g/mol</td>
</tr>
<tr>
<td>7</td>
<td>Garcinone C</td>
<td>(C_{23}H_{26}O_7)</td>
<td>414.45 g/mol</td>
</tr>
<tr>
<td>8</td>
<td>Garcinone D</td>
<td>(C_{24}H_{26}O_7)</td>
<td>428.47 g/mol</td>
</tr>
<tr>
<td>9</td>
<td>Garcinone E</td>
<td>(C_{24}H_{22}O_6)</td>
<td>464.55 g/mol</td>
</tr>
<tr>
<td>10</td>
<td>1-Isomangostin</td>
<td>(C_{24}H_{26}O_6)</td>
<td>410.46 g/mol</td>
</tr>
<tr>
<td>11</td>
<td>9-Hydroxycalabaxanthone</td>
<td>(C_{25}H_{30}O_6)</td>
<td>408.44 g/mol</td>
</tr>
<tr>
<td>12</td>
<td>3-Isomangostin</td>
<td>(C_{24}H_{26}O_6)</td>
<td>410.46 g/mol</td>
</tr>
<tr>
<td>13</td>
<td>6-Deoxy-gamma-mangostin</td>
<td>(C_{23}H_{24}O_5)</td>
<td>380.43 g/mol</td>
</tr>
<tr>
<td>14</td>
<td>BR-xanthone A</td>
<td>(C_{23}H_{24}O_6)</td>
<td>396.43 g/mol</td>
</tr>
<tr>
<td>15</td>
<td>BR-xanthone B</td>
<td>(C_{14}H_{10}O_6)</td>
<td>274.23 g/mol</td>
</tr>
<tr>
<td>16</td>
<td>Garcimangosone A</td>
<td>(C_{28}H_{26}O_6)</td>
<td>460.52 g/mol</td>
</tr>
<tr>
<td>17</td>
<td>1-Isomangostin hydrate</td>
<td>(C_{24}H_{26}O_7)</td>
<td>428.47 g/mol</td>
</tr>
<tr>
<td>18</td>
<td>Calabaxanthone</td>
<td>(C_{24}H_{24}O_5)</td>
<td>392.44 g/mol</td>
</tr>
</tbody>
</table>

**Pharmacokinetics and drug-likeness predictions**
In the present study, we predicted the pharmacokinetic properties and druglike nature of the phytocomponents using the SwissADME web server, Swiss Institute of Bioinformatics, Swiss. We identified gastrointestinal absorption prediction for the oral drug probability according to the white of the BOILED-Egg\textsuperscript{[26]} and Lipinski parameter for the drug-likeness prediction implemented from Lipinski \textit{et al.} (2001)\textsuperscript{[27]}.

\textbf{Biological activity prediction}

We employed PASS (Prediction of Activity Spectra for Substances) web resource as a strong potential tool to predict the biological activity. This web resource estimates the predicted activity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi) as described by Goel \textit{et al.} (2011)\textsuperscript{[28]} and we used Pa>0.3.

\section*{Results and Discussion}

We successfully revealed pharmacokinetics, drug-likeness, biological activity predictions of the various phytocomponents from \textit{Garcinia mangostana} L. All phytocomponents predicted as high in pharmacokinetics prediction (gastrointestinal absorption), except Garcinone E. In addition, all phytocomponents predicted fulfill all Lipinski rule-of-five. Furthermore, 18 phytocomponents of \textit{Garcinia mangostana} L. predicted as antioxidant, anti-inflammatory, and antineoplastic activity.

In addition, phytochemical screening, based on ethnomedicinal data, is considered as an effective approach for the discovery of new therapeutic agents. The major bioactive secondary metabolites of mangosteen are xanthone derivatives\textsuperscript{[1,4]}. The major constituents from the xanthone fraction in mangosteen were found to be α-mangostin and γ-mangostin\textsuperscript{[4,14]}. More than 60 other xanthones are isolated from its different plant parts of including 3-isomangostin, β-mangostin, gartatin, mangostabin, 1-isomangostin, garcinone B, 9-hydroxycalabaxanthone, mangostanol, mangostinone demethylcalabaxanthone, 8-deoxygartanin, and garcinone D\textsuperscript{[1]}. The majority of investigations are focused on the extraction and structure elucidation of xanthones from the pericarp of mangosteen\textsuperscript{[14]}. Recently, the presence of these compounds in the stem, seed, and heartwood was reported by many authors\textsuperscript{[9,11,19,20,23,24,25]}.

On the other hand, bioinformatics provides more efficient target discovery and validation approaches, thus help to ensure that more drug candidates are successful during the approval process and making it more cost-effective\textsuperscript{[26]}. Notably, the work of Lipinski \textit{et al.} analyzed orally active constituents to describe physicochemical ranges for high probability opportunities to be an oral drug. This called rule-of-five delineated the relationship between pharmacokinetics and physicochemical parameters. Lipinski rule-of-five helps in distinguishing between drug-like and non-drug like molecules. It predicts a high probability of success or failure due to drug-likeness for molecules complying with 2 or more of the following rules, such as molecular mass less than 500 Dalton, high lipophilicity, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, molar refraction should be between 40-130\textsuperscript{[27]}.

In this study, an attempt has been made to investigate a more extensive pharmacological appearance of phytoconstituents by application of PASS web resources. The proposed in silico method extends further to generate novel bioactivities of selected phytochemical leads, related side-effects, and their mechanisms. In addition, the recent version of PASS predicts approximately 3750 pharmacological activities, specific toxicities, biochemical mechanisms of action, and metabolic terms on the basis of the structural formula of drug-like substances with average fidelity ~95%. This might be further validated in vitro as well as in vivo trials\textsuperscript{[28]}. In line with this, the present study revealed the use of PASS for exploring the hidden pharmacological potential of the various phytocomponents from the mangosteen.

\section*{Conclusion}

In summary, we revealed the biological activity of 18 phytocomponents of the mangosteen. However, further trials, such as \textit{in vitro} and \textit{in vivo} evaluation, are needed to prove the validity of these findings.

\textbf{Conflict of Interest:} The authors declare that they have no conflict of interest.

\textbf{Source of Funding}: This study supported by the PMDSU Scholarship (Batch III) from the Ministry of Education and Culture of the Republic of Indonesia.
Acknowledgements: Viol Dhea Kharisma (Division of Molecular Biology and Genetics, Generasi Biologi Indonesia Foundation, Indonesia) for the help and cooperation in carrying out this study. We thank EJA, Indonesia, for editing the manuscript. We would like to declare our sympathy to the victims of COVID-19. Tribute goes to the frontliners worldwide, especially in Indonesia.

Ethical Approval: No ethical approval needed.

References

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A Mini-Review of the Medicinal Properties of Okra (Abelmoschus esculentus L.) and Potential Benefit against SARS-CoV-2

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Abstract

Indonesia is rich in medicinal plants, where its population has traditionally used them for generations to cure diseases. Traditional medicine and medicinal plants from Indonesia may lead to the discovery of novel drugs. Abelmoschus esculentus L. or okra is well known as a cure for several diseases and is included in various folk medicinal stockpiles. In recent decades, scientists have discovered the phytochemical composition in the whole okra plant, as well as its pharmacological activities. These studies established the therapeutic potential of okra for drug discovery. Therefore, the present review provides a sneak peek of okra’s pharmacology and phytochemistry, also the potential benefit against SARS-CoV-2.

Keywords: Abelmoschus esculentus L., medicinal properties, SARS-CoV-2.

Introduction

Indonesia is encompassed by many vegetations, including tropical rain forests. Additionally, Indonesia is one of the top five countries in the world that has a high plant diversity, including approximately 6,000 medicinal plants. Consequently, Indonesia is rich in medicinal plants that is used by its population to cure many diseases. One of the medicinal plants in Indonesia is Abelmoschus esculentus L. or okra. Okra is also called qiu kui, bendi, lady’s finger, gumbo, dharos, kacang, and bamieh. It is usually cultivated in warm temperate and tropical regions worldwide and belongs to the Malvaceae family.

Okra is a medicinal plant due to the multiple benefits of its seeds, buds, stems, flowers, leaves, and pods in traditional and contemporary medicine. Okra fruits traditionally have been used as aphrodisiac, appetite-stimulant, cooling, and astringent agents. Other uses of okra include treatment of gonorrhea, bladder blockage, urinary discharges, diarrhea, and chronic dysentery. Okra seeds have also been used as fungicide agents, anti-tumor, and anti-cancer. The pharmacological properties of okra, such as antioxidant, anti-inflammatory, immunomodulatory, gastroprotective, neuroprotective, lipid-lowering, anti-cancer, and anti-bacterial properties (Figure 1). Therefore, the present review provides a sneak peek view into okra’s pharmacology and phytochemistry against SARS-CoV-2.

Okra Distribution

The okra plant grows annually up to 1 m. The plant prefers light (sandy), requires clay soils and well-drained moist soil. The plant prefers basic (alkaline), neutral, acidic soils, and it can grow in very alkaline soil. This plant cannot grow in the shade. The flowers are pollinated by bees and insects and are hermaphrodite (with both male and female organs). The flowers of the okra plant bloom approximately from June to October.

Chemical Composition

The main components of okra bast fiber are lignin, hemicellulose, α-cellulose, and the rest are minor in...
The chemical contexture of okra bast fibers, such as 2.7% aqueous extract, 3.9% fatty and waxy matter, 3.4% pectin matter, 7.1% lignin, 15.4% hemicellulose, and 67.5% α-cellulose.

Nutritional Potential of Okra

Ca, Mg, Na, and K are the important elements in okra pods, which contain approximately 17% seeds; the presence of Ni, Mn, Zn, and Fe has also been reported. A fresh okra pods are low in calories, no fat, high in fiber, and many precious nutrients, including approximately 10-20% folate (46-88 g), about 5% of vitamin A, approximately 30% of the recommended levels of vitamin C (16-29 mg), and both okra mesocarp and seeds are good sources of zinc (80 g/g). Flavonol derivatives (3.4 mg/g of seeds) and oligomeric catechins (2.5 mg/g of seeds) are importantly composed of okra seeds. On the other hand, the mesocarp is structured of quercetin derivatives and hydroxycinnamic acid, 0.3 and 0.2 mg/g of skins, respectively. Seeds and pods are composed in phenolic compounds with notable biological properties such as hydroxycinnamic derivatives, catechin oligomers, and quercetin derivatives. These components, along with the high content of glycoproteins, proteins, carbohydrates, and other dietary elements, enhance the importance in the human diet.

Okra’s Benefits for Human Health

Okra is useful for minimizing blood sugar levels, and therefore beneficial in diabetes, owing to the fiber content, along with other nutritional components. Okra contains high fiber and helps to stabilize blood sugar levels by regulating the rate at which sugar is absorbed. Previous studies have reported that okra polysaccharide possesses hypoglycemic activity and anti-complementary in normal mice. Additionally, okra can be used in kidney disease prevention.

The okra pods are high in beta-carotene and vitamin A, which are important for healthy skin and sustaining eyesight. Okra is used to rectify eyesight. Additionally, these types of nutrients also assist in inhibiting skin related problems and eye-associated diseases.

The soluble fiber within okra helps to reduce serum cholesterol. Okra is also loaded with pectin, which can help in reducing high blood cholesterol. Consuming okra is an efficient method for managing the body’s cholesterol level. Okra used to improve heart health.

Okra is a good vegetable for those exhausted and feeling weak. Okra is also used in asthma, irritable bowel lung inflammation, ulcers, and sore throat.

Okra used to treat many digestive system problems. Okra is used to support colon health. The polysaccharides present in okra pods suggested that they help to erase the adhesive between stomach tissue and bacteria, preventing bacteremia. Okra’s polysaccharides are effective at inhibiting the adhesion of Helicobacter pylori. Therefore, okra can create an environment that prevents destructive cultures from flourishing and cleanse our stomach. Okra smoothly moves down the colon, excess water in its path and absorbing toxins.

Mucilage are very rich in okra roots and have a sturdily demulcent action. This mucilage can be used as a plasma replacement. The roots infusion is used in the medication of syphilis. In Nepal, the juice of the roots is used to treat boils, wounds, and cuts. In addition, the leaves furnish an emollient poultice. Then, a decoction of the immature capsules is used as diuretic, emollient, and demulcent. It is used in the medication of dysuria, gonorrhea, and catarrhal infections. The seeds of okra have stimulant properties and anti-spasmodic. On the other hand, infusion of the okra roasted seeds also has specific properties. Based on these numerous beneficial properties, okra may be a viable candidate against the SARS-CoV-2, but we need many confirmatory studies such as in silico, in vitro, and in vivo (Figure 1).
Medicinal plants for a forthcoming cannot bring any cognizance for any antagonistic effects from the utilize of plants. In addition, always seek recommendation from professionals before using a plant medicinally for any of its properties including vulnerary, stimulant, emollient, diuretic, diaphoretic, demulcent, and antispasmodic properties.

**Conclusion**

In summary, prior potency and scientific reports of the medicinal properties of okra identify it as a valuable plant and establish it as a candidate for future development of drugs against SARS-CoV-2.

**Conflict of Interest:** The author declare that they have no conflict of interest.

**Source of Funding:** This study supported by the PMDSU Scholarship (Batch III) from the Ministry of Education and Culture of the Republic of Indonesia.

**Acknowledgements:** We thank Editage for editing the manuscript. We would like to declare our sympathy to the victims of COVID-19. Tribute goes to the frontliners worldwide, especially in Indonesia.

**Ethical Approval:** No ethical approval needed.

**References**


Identify Population Attitude Towards Osteoporosis and Pharmacists Involvement in Community Education in Makkah, Saudi Arabia: A Cross-Sectional Study

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Abstract

Introduction: Fracture is the most severe clinical consequence of osteoporosis. Unfortunately, most patients are unaware that they have fragile bones until they fracture the hip, spine, pelvis, or wrist. Thus, increase disability, hospitalization, and so on total health care costs. In Saudi Arabia, many middle-aged and older women were unaware of osteoporosis risk factors with a high prevalence of osteoporosis reported in Saudi Arabia.

Aim: This study’s main objective is to evaluate population awareness about osteoporosis and pharmacist’s involvement in patient education to minimize the risk of osteoporosis in the Makkah region.

Methods: A cross-sectional study was performed using an online survey. A total of 1390 participants completed the questionnaire from 15 February to 15 March 2019.

Results: Only 1.4% of participants had a risk for osteoporosis and got the advice from pharmacists. Significantly, 53.7% had a chance but didn’t get any advice from either physicians or pharmacists.

Conclusion: The results show a low self-awareness level in Makkah’s general population with insufficient involvement of pharmacists in patient education.

Keywords: osteoporosis, pharmacists, fracture, vitamin D deficiency, and calcium

Introduction

Several definitions of osteoporosis have been offered to describe fragility fractures; the process is characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and increased fracture risk. Fracture is the most severe clinical consequence of osteoporosis. Unfortunately, most patients are unaware that they have fragile bones until they fracture the hip, spine, pelvis, or wrist. Thus increase disability, hospitalization, and so on total health care costs. The diagnosis of osteoporosis can be determined by measuring bone mineral density (BMD). According to The World Health Organization, osteoporosis is diagnosed with a T-score of < -2.5. Non-modifiable risk factors for osteoporosis include female gender and old age. In contrast, modifiable risk factors for osteoporosis have calcium intake, vitamin D deficiency, smoking, alcohol intake, exercise, underlying disease conditions such as rheumatoid arthritis, and systemic lupus erythematosus. Some medications have been reported to increase osteoporosis, such as glucocorticoid treatment, levothyroxine, proton pump inhibitors, and warfarin.

Osteoporosis treatment medications include bisphosphonates, receptor activator of nuclear
factor kappa-B ligand inhibitors, estrogen agonists/antagonists, parathyroid hormone analogs, calcitonin, Bisphosphonate, and Ca and Vit D supplement.\(^\text{6, 14}\).

Available treatment does not always solve the problems as several studies indicated the link between the long-term use of bisphosphonates and the risk of fracture of the femur.\(^\text{15, 16}\)

Osteoporosis is estimated to affect 200 million people worldwide.\(^\text{17}\) However, many studies reported that incidence is varied among populations.\(^\text{18-21}\) It affects 30% of all postmenopausal women in the United States and at least 40% in Europe.\(^\text{22}\) It affects men, as well.\(^\text{23-25}\) In Saudi Arabia, osteoporosis reported prevalence ranges from 30% to 48% in women,\(^\text{26-32}\), and 21% of Saudi men older than 50\(^\text{33}\). Importantly, conducted studies in Riyadh showed a low level of awareness about the disease among Saudis men and women.\(^\text{34, 35}\) Osteoporosis is considered as one of the most important causes of morbidity and mortality in aging women.\(^\text{36}\)

Prevention is better than cure, and awareness about this disease can help in its prevention.\(^\text{37}\) Many studies related to osteoporosis have been conducted in different regions worldwide and pointed out different awareness levels with poor knowledge about this disease in some countries such as UAE, India, and the USA.\(^\text{34, 38-43}\)

Until our knowledge, no previous study has been conducted to assess pharmacists’ involvement in patient education about osteoporosis. Thus, we aim to evaluate population awareness about osteoporosis and pharmacists’ involvement in patients’ education to minimize the risk of osteoporosis in Makkah region.

Methodology

Study design

This is a cross-sectional study with a total of 1390 participants who completed the questionnaire from February 15 to March 25, 2019 within Makkah, Saudi Arabia.

Data collection

The online survey was anonymous, prepared in Arabic-language format. Consent of participants was considered by their submission. The questionnaire had four sections; demographic characteristics and risk factors of osteoporosis, daily food intake habits of participant in relation to bone health, participant attitude towards disease management and involvement of physicians and pharmacist in patient education.

Statistical Analysis

All the variables were analyzed using SPSS Var 23.0 software 2015. Descriptive analyses such as percentages and graphs were used to describe the findings of this study.

Results

Participants included in this study were predominantly female (74%, n=1028). Overall 58% of participants had moderate diet contain dairy products and other calcium sources. Beside 31% had low diet containing dairy products and other calcium sources. However, only 11.9% had high diet contain dairy products and other calcium sources (Figure.1. A). Among our participants, 5% of participants are taking calcium supplements (Figure. 1. B). While 13% are taking vitamin D supplements (Figure. 1. C).

Regarding participants attitude towards osteoporosis and its risk factors, around 87.1% of participants never had check for osteoporosis or monitor level of calcium supplements (Figure. 2. A). Despite 66% had at least one of diseases or medications that may increase the risk of osteoporosis (Figure. 2. B). Specifically, 52.7% of participant had a risk for osteoporosis but didn’t check for diagnosis. Additionally, 7.3% didn’t remember if they ever checked for osteoporosis or not even though they have risk factor (Table. 1).

Finally, only 1.4% of participants had risk for osteoporosis and got the advice from pharmacists. Furthermore, 7.5% listen to advice from a doctor and 2.9% listen from both. Importantly, 53.7% had a risk but didn’t get any advice from either physicians or pharmacist (Table. 2).

Discussion

This questionnaire-based study helps to evaluate the patient attitude towards osteoporosis and pharmacists involvement in patients education to minimizing risk of osteoporosis in Makkah region. Current study indicates
that lifestyle patterns of most participants could increase their risk of osteoporosis. Most of participants 89% had low or intermediate calcium intake compared 60% in previous study conducted in Riyadh. It is very important to maximize calcium daily intake in order to increase bone mass, thereby minimize the risk of fracture and osteoporosis in the future. Furthermore, our data indicate many peoples may suffer from osteoporosis but they didn’t know, as their risk factors suspect potential of osteoporosis disease. Previous study conducted by Al-sharhrani focused on awareness of postmenopausal women.(35) However, this study is more generalized since we included younger women too.

For the first time, this study found low level of pharmacist involvement in patient education about osteoporosis even in patients with some risk factors. Health authorities and pharmacists should have better involvement in patient education about osteoporosis. Efforts should be applied to improve the awareness about the disease and the importance of early follow up and therefore minimize risk factors and cost of osteoporosis management.

Future studies including another research questionnaire that includes pharmacists to take their opinion, may be helpful. As the pharmacist may have good counselling and communication skills but the patient’s response is poor. In that case, increase the awareness of population about the role of pharmacists in community is needed.

**Conclusion**

The results show low self-awareness level in general population in Makkah with poor involvement of pharmacist in patient education.

Figure 1. Participant’s daily habits towards factors affecting osteoporosis: (A) The extent to which daily food intake contains dairy products. (B) Percentage of participants who take calcium supplements. (C) Percentage of participants who take vitamin D supplements
Figure. 2. Participants risk factors and their attituded towards diagnosis: (A) Percentage of participants who checked for osteoporosis or calcium level. (B) Evaluation of risk factors of osteoporosis among participants.

Table. 1: Percentage of osteoporosis checking among participants.

<table>
<thead>
<tr>
<th>Having at least one risk factor of osteoporosis (disease or drug)</th>
<th>Checking for osteoporosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>431 (31%)</td>
<td>7 (0.5%)</td>
</tr>
<tr>
<td>Yes</td>
<td>733 (52.7%)</td>
<td>78 (5.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>1164 (83.7%)</td>
<td>85 (6.1%)</td>
</tr>
</tbody>
</table>
Table. 2: Pharmacists involvement in patients’ education about risk of osteoporosis.

<table>
<thead>
<tr>
<th>Having at least one risk factor of osteoporosis (disease or drug)</th>
<th>I did not receive any advice</th>
<th>Receiving advice from pharmacist</th>
<th>Receiving advice from physician</th>
<th>Receiving advice from both</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>434 (31%)</td>
<td>8 (0.6%)</td>
<td>13 (0.9%)</td>
<td>23 (1.7%)</td>
</tr>
<tr>
<td>Yes</td>
<td>747 (53.7%)</td>
<td>20 (1.4%)</td>
<td>104 (7.5%)</td>
<td>41 (2.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>1181 (85%)</td>
<td>28 (2%)</td>
<td>117 (8.4%)</td>
<td>64 (4.6%)</td>
</tr>
</tbody>
</table>

**Conflict of Interest:** The authors have not declared any conflict of interests.

**Ethical Clearance:** Ethical approval was obtained from IRB comity at College of Medicine, Umm Al-Qura University.

**Source of Funding:** No funding available for this study.

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The Possible Relation between Pityriasis Alba and Intestinal Parasitic Infestation Among Children in Tikrit City, A Case Control Study

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Abstract

Background: There is a solid belief stated that the appearance of hypopigmented patches on children face is a sign of their helminthic or parasitic intestinal infestation despite the constant health education about the absence of such relation. Pityriasis Alba (PA) is common benign skin lesion characterized by fine scaly hypopigmented patches (HP) or macules¹ mostly in the face and upper body parts.²⁻⁴ PA may be atopic dermatitis related or endemic PA. PA occurs mostly in 3-16 years old children and accounts of 5% of pediatric population worldwide. Prevalence rate in Iraq is high (38.2%). No specific cause of PA has been identified. Diagnosis of PA depends on the clinical picture and treatment may implicate sunscreen and topical corticosteroids despite its self-limiting privilege. Helminthiasis (worm infestation) is the hosting of parasitic worms after invading humans and other animals necessarily to complete their lifecycle. *Enterobius vermicularis* is the most common helminthic intestinal infestation among children in Iraq. The main complaint of helminthiasis is the perianal itching, especially at bed time. The study aims to determine the relation of intestinal helminths with PA. Study Design: This prospective and a case-control study had consisted of 43 males (53.5%) and females (46.5%) children of 5-15 years of age presented with HP. Results and Discussion: 4 (9.3%) cases presented with *Enterobius vermicularis* given antihelminthic drugs and had their infestation eradicated completely after a week. 39 (90.7%) children presented with no infestation, regarded as control group, and so given placebo treatment. Number and size of HP varied after the 6 weeks of treatment. Although cases shown more numerous (6.0 ± 2.9) HP and larger size (120.0 ± 72.2) than control group (4.9 ± 3.6 and 90.0 ± 58.3 respectively), but the difference was not significant for both groups (cases and control) and between them for both HP number and sizes (p>0.6). Conclusion: There is no enough prove of the relationship between children intestinal parasitic infestation and the appearance of hypopigmented patches.

Keywords: pityriasis alba, erythema streptogenes, pityriasis streptogenes, impetigo furfuracea, pityriasis simplex, parasites, *Enterobius vermicularis*, pinworm, threadworm, seat worm, nematode, roundworm.

Introduction

Pityriasis Alba (PA) is common benign skin lesion or dermatological disorder characterized by the appearance of fine scaly hypopigmented patches (HP) or macules¹ that are most commonly seen in the face, neck, shoulders, trunk,² and to a lesser extent, in extremities²⁻⁴. There are two main (typical) types of pityriasis alba and two atypical types:

1. Atopic Dermatitis Related PA, is mostly related with postinflammatory hypopigmentation. Most of the patients were cases or have a history of atopy, and PA in this case regarded as an atopic dermatitis minor manifestation.

2. Endemic PA, Endemic PA is usually occurring among infants to children of low socioeconomic condition.⁶

3. Pigmented Pityriasis Alba, mostly described as a central darker patch surrounded by a lighter
colored scaly zone of PA. Pigmented PA lesion have a bluish hue in the center that is surrounded by a halo of hypopigmentation.

4. Extensive PA (Progressive Macular Hypomelanosis). The lower part of the trunk is the most common site of involvement symmetrically and usually in a relapsing attitude. Extensive PA patches are widespread and have more persistent course. Lesions aren’t favoring the face. Females are more often affected than males. (7-8)

There is no gender difference for the disease. There is a global distribution of PA, though its prevalence may differ among different countries. The most prevalent countries were Iraq (38.2%), India (31%), Mali (20%), and Egypt (18%); while lower prevalence was in developed countries like United states (5%) and Hong Kong (1%). PA occurs more predominantly in children between the ages of 3-16 years. PA is noninfectious and there is no identified peculiar etiology. Many cases with PA are presented with iron and copper deficiency. Possible triggering factors that may cause PA are deficiency of vitamins & calcium, temperature variations, humidity, excessive sunlight exposure, frequent bathing, usage of harsh detergents and soaps, dry and itching skin, hypopigmentation, worms and parasites, stress, deficiency of copper and atopic diseases and/or a family history of eczema (2-3).

The diagnosis in most cases of pityriasis alba is straightforward and depending on the clinical picture (3). PA most frequently seen as 2 or 3 macules or patches in different stages. Vargas et al described 3 stages of PA:

1. The early stage, also called papular erythematous stage, begins as faint pink to red elevated bordered round to oval macule or patch that may last for weeks. In most cases this erythematous stage may run unnoticed.

2. The next intermediate stage, papular hypochromic stage, or follicular pityriasis alba. The patch is converted into a smooth scaly layer.

3. The final stage, the smooth hypochromic stage presented as a visible, round and hypopigmented macule with mostly well-defined borders. In this stage, the patient usually or his/her parents will seek medical assistance (10).

Telling the patients and/or their parents about the PA benign nature and self-limited course is mandatory. They should be informed about its slow resolution that may exceed a one year (1). Patients can be informed to follow some lifestyle modification with support use of sunscreens, skin moisturizers, and skin hygiene. Topical steroids (low-potency) appear to be more widely prescribed.

**Intestinal Parasites:**

According to the given hypothesis, this study emphasizes on helminthic infestation. Among the most common modes of transmitting these organisms is through contaminated water, food, soil, as well as contact. Helminthiasis (worm infestation) is the hosting of parasitic worms after invading humans and other animals necessarily to complete their lifecycle and either causing clinical manifestation or hide as an asymptomatic carrier status. In third world countries, including Iraq, the majority of helminths infections are associated with poor sanitary facilities, indiscriminate disposal of human waste, inadequacy and lack of quality drinking water. It is also potentiated by poverty and low socioeconomic status. *Enterobius vermicularis* or the so-called pinworm, threadworm, or seatworm, is a nematode (roundworm) that is common in human children transmitted by feco-oral route. It hosts humans only (11).

Night perianal itching grew the suspicion of the worm infestation. Eggs can be recovered using the “Scotch Tape” technique in the morning before a bowel movement. Transparent Scotch Tape is applied directly to the perianal area, and then placed on a microscope slide for examination. Eggs are football shaped and have an outer shell. Infectious larvae are often visible inside the egg. The small adult worms may be seen in a stool test (ova and parasites). Because the eggs are lightweight and highly infectious, it is important for bed linens, towels, and clothing to be washed in hot water to prevent reinfection (5).

**Patients and Methods**

This was a prospective and a case-control study conducted in the department of dermatology of Salahuldin-General Hospital in Tikrit city, Salahuldin province, Iraq. The study was conducted during the
period from Nov 2019 to May 2020. The study targeted children aged between 5 and 15 years. A total of 43 children were enrolled in the study. The intestinal parasite infested attendant children presented with skin hypopigmented patches were regarded as the case group. The non-infested children with PA were the control group. All participants passed a medical examination by a dermatologist and consolidated by taking opinions of two or more dermatologists. Three separated (2-days apart) stool samples were taken for parasitological examination from all the participants. Additionally, stool samples and sticker tapes were taken from PA children with parasitic infestation a week after treatment completion to confirm parasite elimination. No local drug applications, sunblock, or soothing agents were given for HP. Therapy was given to PA patients infected with Enterobius vermicularis with mebendazole (a single 100 mg chew tablet that can be repeated 3 weeks later if infestation had not been eradicated)\(^{(12)}\), while parasite free PA children were given placebo made of starch pills of the same size and shape of the mebendazole tablet, and given to the control group in the same amount and frequency. Both treatment and placebo were well tolerated, and no side effects were complained. All patients were examined for the pityriasis alba at the first visit. After a one week another contact with the patient to evaluate the state of helminthic eradication. After 6 week patients were contacted to observe the changes in skin hypopigmentation. Clinical efficiency of parasite elimination was evaluated one week after the completion of antiparasitic therapy. A positive clinical response included: complete HP disappearance and reduction of intensity, size and/or hue of HP. A negative clinical response included: the absence of visual changes of HP or enlargement of size and hue of HP\(^{(13)}\).

**Results**

The forty-three studied children ages were ranged from 5 to 15 years. Their mean age was 9.2 years (Table 1). It was consisted of 23 (53.5%) males and 20 (46.5%) females. Twenty-eight of them were living in urban districts (65.1%) whereas 15 were living in rural areas (34.9%). The HP presented in different numbers, sizes, and various body regions (Table 2). Four (9.3%) children were shown to have parasitic intestinal infestation, while 39 (90.7%) children with HP were free of infestation. All infesting parasites were the helminths *Enterobius vermicularis* that were symptomatic and had been isolated and observed microscopically (Figure 1).

<table>
<thead>
<tr>
<th>Demography of the studied sample.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Range</strong></td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
</tr>
<tr>
<td><strong>5-15</strong></td>
</tr>
<tr>
<td><strong>Gender Number (%)</strong></td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>23 (53.5%)</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>20 (46.5%)</td>
</tr>
<tr>
<td><strong>Residence Number (%)</strong></td>
</tr>
<tr>
<td>Urban</td>
</tr>
<tr>
<td>28 (65.1%)</td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>15 (34.9%)</td>
</tr>
</tbody>
</table>

SD: Standard Deviation

<table>
<thead>
<tr>
<th>Hypopigmented patch distribution and sun exposure.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypopigmented Patch</strong></td>
</tr>
<tr>
<td><strong>Range</strong></td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
</tr>
<tr>
<td><strong>Number</strong></td>
</tr>
<tr>
<td>1-18</td>
</tr>
<tr>
<td>5.0 ± 3.5</td>
</tr>
<tr>
<td><strong>Sum of size (mm)</strong></td>
</tr>
<tr>
<td>20-325</td>
</tr>
<tr>
<td>92.8 ± 59.3</td>
</tr>
<tr>
<td><strong>Average size (mm)</strong></td>
</tr>
<tr>
<td>12-50</td>
</tr>
<tr>
<td>20.7 ± 6.5</td>
</tr>
<tr>
<td><strong>Duration (months)</strong></td>
</tr>
<tr>
<td>1-36</td>
</tr>
<tr>
<td>10.7 ± 8.7</td>
</tr>
<tr>
<td><strong>Sun exposure (hour/day)</strong></td>
</tr>
<tr>
<td>1-8</td>
</tr>
<tr>
<td>4.5 ± 2.4</td>
</tr>
<tr>
<td><strong>Family members</strong></td>
</tr>
<tr>
<td>1-4</td>
</tr>
<tr>
<td>Median = 2</td>
</tr>
</tbody>
</table>

SD: Standard Deviation
HP distributed in different parts of the body. Ninety-three percent of children had HP in the cheeks and to a lesser extent in forehead and neck 70% and 67% respectively. This study had shown HP presented in shoulders in 19%, upper extremities in 33%, and in the trunk in 9% of cases.

Figure 1. Ova and a helminth of Enterobius vermicularis as shown under light microscope in the positive infested studied cases.

None of cases observed in the study had their HP eliminated completely after 6 weeks of treatment (Figure 2). The study shown 14 (32.56%) children of fixed size HP after this period and the rest were underwent change in their HP size as shown in Table 3.

Table 3. Destiny of HP change after six weeks of treatment or placebo.

<table>
<thead>
<tr>
<th>HP Size Change</th>
<th>Infested</th>
<th>Non-infested</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disappear</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Reduced</td>
<td>75%</td>
<td>46.15%</td>
<td>48.84%</td>
</tr>
<tr>
<td>Fixed</td>
<td>0%</td>
<td>35.90%</td>
<td>32.56%</td>
</tr>
<tr>
<td>Enlarge</td>
<td>25%</td>
<td>17.95%</td>
<td>18.60%</td>
</tr>
</tbody>
</table>

HP: hypopigmented patch
Cases present with an average of 6 hypopigmented patches in the first visit as well as in six weeks after treatment, while control group shown HP average number of 4.9 in the first visit and 4.8 six weeks after placebo (Table 4). The HP size in infested and control groups were summarized in Table 4.
Table 4. Hypopigmented patch number and size in infested compared to non-infested children in the first visit (V1) and six weeks after treatment (V2).

<table>
<thead>
<tr>
<th>HP</th>
<th>Infested (Mean ± SD)</th>
<th>Non-infested (Mean ± SD)</th>
<th>Total (Mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>6.0 ± 2.9</td>
<td>4.9 ± 3.6</td>
<td>5.0 ± 3.5</td>
<td>0.7642</td>
</tr>
<tr>
<td>V2</td>
<td>6.0 ± 3.6</td>
<td>4.8 ± 4.1</td>
<td>4.9 ± 4.0</td>
<td>0.7718</td>
</tr>
<tr>
<td>change</td>
<td>0.0 ± 2.8</td>
<td>-0.1 ± 1.9</td>
<td>-0.1 ± 2.0</td>
<td>0.9602</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sum (mm) in V1</td>
<td>120.0 ± 72.2</td>
<td>90.0 ± 58.3</td>
<td>92.8 ± 59.3</td>
<td>0.61</td>
</tr>
<tr>
<td>sum (mm) in V2</td>
<td>111.3 ± 89.2</td>
<td>86.8 ± 78.9</td>
<td>89.1 ± 79.0</td>
<td>0.7566</td>
</tr>
<tr>
<td>change (mm)</td>
<td>-8.8 ± 69.8</td>
<td>-3.2 ± 39.7</td>
<td>-3.7 ± 42.2</td>
<td>0.8886</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.9044</td>
<td>0.9522</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HP: hypopigmented patch, SD: Standard Deviation, p: probability (p>0.05: not significant), mm: millimeter.

**Discussion**

In Iraq, there is a common belief among society regarding the presence of white patches in the face of the child as a sign of intestinal infestation. Lower than a tenth (9.3%) of children with hypopigmented patch complaining of intestinal parasitic infestation is not enough evidence as a causation for the majority (90.7%) of HP incorporated in the study were found to be free infestation. A near finding was obtained by Vinod et al (2012) whom microscopic examination of the stool shown ova presented in 15.5% of the sample, and concluded no significant relationship between intestinal parasitic infestation and HP (14). Unlike, Osipova (2017) founded 43.4% out of 30 children with HP had helminthic intestinal infestation and concluded a positive significant relationship between infestation and HP (15). Three out of the four infested children in this study were living in rural area. This may be due to the lower hygiene, lower sanitation, habitual longer contact with soil, or eating unclean vegetables or fruits.

Similar to what found by Vinod et al (14) and Toychiev et al (16), the majority of children in this study had HP in the head especially in cheeks (93%), forehead (70%), and in the neck (67%) (14, 16). The study shown those children with intestinal infestation had slightly more numerous HP compared with control group (mean = 6.0 and 4.9 respectively) (Table 4). This difference was not statistically significant (p=0.7642); nor significant was the difference between the number of HP observed after 6 weeks of taking antihelminthic vs placebo treatment (p=0.7718). This was agreeing with Vinod et al (14) whom found a similar non-significant relation; but disagreeing with both Toychiev et al and Osipova. This study shown no significant change in number of HP after 6 weeks of the treatment or placebo for both intestinally infested children (p=1) and control group (p=0.976). This was disagreeing with what was found by Toychiev et al whom declared that 33.3% of children with enterobiasis had complete disappearance of their HP after 6 weeks of treatment and had their parasitic infestation eliminated. It is also disagreeing with Osipova whom founded 69.2%
of cases underwent complete disappearance of their HP after 6 weeks of antihelminthic treatment (compared to 0% in this study (Table 3).

In this study, children with intestinal helminthic infestation shown larger HP size (measured by mean of the summation of diameters of patches for each child) compared with control group (120 and 90 mm respectively) (Table 4). This difference was not statistically significant (p=0.61). Similar insignificant difference was found between case vs control after treatment and placebo (p=0.7566). Although there was some decline in the HP size of the group of cases after treatment (averaged 8.8 mm reduction), it was also statistically not significant (p=0.9044). A lesser (3.2mm) (but also insignificant p=0.9522) decline in the average of HP size of control group after 6 weeks of placebo intake. This decline was observed in 21 (48.84%) (i.e. nearly a half) of the total 43 observations. These finding were disagreeing with Toychiev et al whom founded 20.3% of patients had reduced HP size significantly.

Conclusions

1. Despite the familiarity of the relation between intestinal helminthic infestation and HP among the society, there is no significant relationship between the two.

2. Despite the frequently observed cases of intestinal infestation attending pediatrics consultation as well as children with HP attending dermatology’s one; there is no prove stating the former as an aetiology of the latter.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References


Investigate the Therapeutic effect of Autologous Adipose-Derived Stem Cells (ADSCs) on Ischemic Renal Failure in Dogs

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Abstract

Background/ aim: The kidney excretes waste materials and regulates important metabolic functions, and renal disorders constitute a significant medical problem and can result in fatalities. In the present study, mesenchymal stem cells derived from canine adipose tissue were isolated and evaluated for their ability to improve renal function in a canine model of ischemic renal failure. Materials and Methods: The canine ischemic renal failure model was developed by a traumatic occlusion of the renal artery in 10 dogs. ASCs were administered directly into the renal artery following ischemic renal failure induction. Blood analysis and histological parameters were analyzed. Results: The group treated with ASCs had decreased blood urea nitrogen and creatinine levels, and showed an improving histological manifestation. ASCs were detected around the tubules of these kidneys at the histological level. Conclusion: our findings suggest that ASCs could be an alternative therapeutic agent for canine ischemic renal failure.

Key words: dog, ischemic renal failure, autologous adipose-derived stem cells

Introduction

Acute renal failure (ARF) is a type of kidney disorder where epithelial cells of the renal proximal tubule in the nephron undergo necrosis as a result of ischemia or toxic damage. This leads to sudden decrease in glomerular filtration rate (GFR), which is caused by loss of auto-regulation, tubular obstruction, and increased renal vasoconstriction (¹). In kidney failure, the therapeutic strategies of renal replacement are still more sufficient than those of current hemodialysis (²). The adipose tissue has recently been shown to be involved in the pathophysiology of renal disease and kidney failure (³). The main cause of renal failure in dogs is renal ischemia. Usually renal failure caused by renal nephrotoxic substances, infection, renal ischemia, inflammation, shock or hypovolaemia and obstruction of urinary tract by calculi, strictures or tumors (⁴,⁵). Adipose-derived stem cells (ASCs) are confirmed as a source of multipotent stem cells that can be differentiated into osteogenic, chondrogenic, myogenic, and adipogenic cells in the presence of lineage-specific induction factors in vitro (⁶). The major cause is tubular necrosis as a consequence of ischemic renal injury after episodes of hypotension or surgical vascular clamping (⁷). The pathogenic incidents in ischemia/reperfusion damage include apoptosis and acute tubular necrosis, glomerular damage, and inflammation. Healing of inflammatory and ischemic renal injury include mesangial, epithelial and endothelial, regeneration (⁸). Mesenchymal stem cells have the ability to divide, generate and differentiate, which at last is part of either an adult or embryonic tissue (⁹). It has tremendous
potential for the evolution of therapies at the future (10). Mesenchymal stem cells can secrete growth factors and cytokines such as vascular endothelial growth factor (VEGF), insulin like growth factor-1 (IGF-1), hepatocyte growth factor (HGF) and anti-apoptotic cytokines which have capability to diminish the fibrosis of tissues through regeneration and play a role in tissue repair (11, 12). MSCs have capability to differentiate into three types of cells such as chondrocyte, osteocytes, and adipocytes. Mesenchymal stem cells are immunosuppressive and non-immunogenic, and have the capability to migrate to sites of damaged tissue and inflammation to share in tissue healing (10, 13).

Adipose tissue is a more eligible source because of its plenty and rapid expansion rate in culture. Subcutaneous adipose tissue usually composed of mature adipocytes and a heterogeneous stromal vascular fraction which involve fibroblasts, pre-adipocytes, endothelial cells, vascular smooth muscle cells, monocytes, lymphocytes and adipose stem cells. Adipose stem cells are promising candidates for cellular therapy in regenerative medicine due to its properties such as anti-inflammatory, anti-apoptotic, proangiogenic, anti-scarring and immunomodulatory effects (14). Angiogenesis based on the pathology of ischemic diseases, it is reasonable to believe that angiogenesis and vasculogenesis are the indispensable premises for tissue regeneration (15). The purpose of the study reported here was to investigate the effects of autologous adipose stem cells on renal function and renal structural changes, in dogs.

Materials and Methods

Ten healthy dogs age range, 1 to 1.5 years; weight ranges, 15 to 18 kg were used in the study. It divided into two equal groups. Prior to the experiment, all dogs were acclimated for at least two weeks and screened for underlying diseases on the basis of results of a complete blood count CBC and serum biochemical analysis. During the experiment, dogs were housed separately in cages and fed dry food and water.

Evaluation of renal function: Renal function was evaluated based on blood urea and creatinine levels. Blood samples were collected from cephalic vein from each dog three days before surgery, three days after surgery and same period after treatment with ASCs to determine the serum chemistry profile. The reference range of serum BUN was 8-30 mg/dl and that of serum creatinine was 0.5-1.5 mg/dl, Serum BUN and creatinine levels above the reference range were considered abnormal.

Collection adipose tissues: Briefly, adipose tissue was obtained from subcutaneous, inguinal fat depots of dogs, using standard surgical procedures. At obtaining, adipose tissue was placed into a sterile 50 mL conical tube containing 15 mL of phosphate-buffered saline (PBS) and brought quickly to stem cells laboratory.

Cell culture and characterization:

Collected adipose tissue was washed three times with phosphate-buffered saline containing 100 IU/mL penicillin and 100 g/mL streptomycin, then chopping sample with scalp knife size 15 and surgical forceps and digested for 1 hr. at 37 ºC with collagenase type IA. The enzymatic activity was inhibited with (DMEM) Dulbecco’s Modified Eagle’s Medium containing 10% (FBS) fetal bovine serum. Following centrifugation at 1200 x g for 5 min, the pellet was filtered through a 70 µm falcon cell strainer to remove debris, then incubated in DMEM containing 10% FBS at 37˚C in a humidified atmosphere of 5% CO₂ after 48 hrs. cultures were washed with PBS to remove non-adherent cells and incubated with fresh medium, which was changed every 48hrs. until cells reached 70% to 80% confluence. The cells were then repeatedly subculture under standard conditions (4). Cells were brought to a final volume of 2mL with PBS and loaded into a sterile syringe for injection.

Induction of unilateral renal ischemia reperfusion (I/R):

After standard surgical preparation performing, Dogs were anesthetized by intramuscular injection mixture of ketamine HCL (15 mg/kg) and 2% xylazine (5 mg/kg). A midline abdominal incision was made to expose the left kidney. Blood supply to the kidney was interrupted by clamping the left renal artery using an artery clamp for 1 hour. After 1 hour the clamp was removed and reperfusion was confirmed visually. Then the catheter was inserted into the renal artery, while its proximal end inserted through a tunnel created in the dorsal wall of the abdominal cavity and fixed to the skin out the body with silk stitch suture and closed.
its opening permanently. The wound layers were then closed routinely and the animals were allowed to recover with free access to food and water (16).

**Intra-arterial ASCs infusion:**

At day 3, after inducing ischemic renal failure, all dogs placed under general anesthesia, and ASCs were injected directly into the left renal artery via a catheterization approach. A total of \(10^6\) ASCs suspended in \(2\) ml phosphate-buffered saline was slowly infused through the arterial catheter into the kidney over \(1–2\) minutes in treated group while control group injected with normal saline.

**Histopathological examination:**

At day 10 of ischemic renal failure induction (day 7 of treatment with normal saline and ASCs infusion), the kidneys were harvested and fixed with \(10\%\) buffered formalin. The fixed tissues were embedded in paraffin wax and \(4-\mu\)m sections were stained with hematoxylin and eosin (H&E) by standard procedures for histological examination.

**Statistical analysis:** Renal function analyzed using SPSS Statistics. Statistical analysis of serum BUN and creatinine was performed by one way ANOVA for comparisons among groups. P values of \(p < 0.05\) were considered to record statistical significance. All data were expressed as means ± standard error (SE).

**Results**

**Evaluation of renal function:**

The results showed that the levels of serum blood urea nitrogen (22.70 ± 0.54 and 14.40 ± 1.67) and creatinine (0.93 ± 0.10 and 0.77 ± 0.10) in both groups at 3 days before induction ischemic renal failure was with in normal reference range. At 3 days after induction ischemic renal failure the results showed increase significant differences (\(P< 0.05\)) in the levels of BUN (36.80 ± 0.63 and 33.90 ± 1.27 in control and treated groups respectively) and creatinine (2.70 ± 0.10 and 2.71 ± 0.03 in control and treated groups respectively) compared with the levels at 3 days before surgery. While the levels of BUN and creatinine at 7 days after treatment showed continuous increasing in the group which treated with normal saline, but significantly decreased in the ASCs treated group. (Tab.1, Tab.2)

<table>
<thead>
<tr>
<th>Time/days</th>
<th>Groups</th>
<th>3 days before surgery</th>
<th>3 days after surgery</th>
<th>10 days after surgery (7 days after treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.93 ± 0.10 A a</td>
<td>2.70 ± 0.10 B b</td>
<td>3.86 ± 0.09 C c</td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>0.77 ± 0.10 A a</td>
<td>2.71 ± 0.03 B b</td>
<td>1.78 ± 0.03 C d</td>
<td></td>
</tr>
</tbody>
</table>

Similar capital letters horizontally denote no differences \(P<0.05\) among periods.
Different small letters vertically refers to the existence of significant differences $P<0.05$ among groups.

**Table-2: Shows Mean Values of blood urea nitrogen (BUN) mg/dl.**

<table>
<thead>
<tr>
<th>Time/days Groups</th>
<th>3 days before surgery</th>
<th>3 days after surgery</th>
<th>10 days after surgery (7 days after treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.70 ± 0.54</td>
<td>36.80 ± 0.63</td>
<td>39.87 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>A a</td>
<td>B b</td>
<td>B c</td>
</tr>
<tr>
<td>Treated</td>
<td>14.40 ± 1.67</td>
<td>33.90 ± 1.27</td>
<td>30.20 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>A b</td>
<td>B b</td>
<td>B d</td>
</tr>
</tbody>
</table>

Similar capital letters horizontally denote no differences $P<0.05$ among periods.

Different small letters vertically refers to the existence of significant differences $P<0.05$ among groups.

**Histopathological examination:**

Control group at day 7 of treatment with normal saline infusion showed, atrophied glomeruli as well to periglomerular necrosis of renal tubules, in addition to marked dilation of some renal tubules. Hyperplasia of mesengial cells, as well to vacuolation of mesengial cells of the glomerulus, in addition to necrosis of peri-glomerular renal tubules with infiltration of inflammatory cells (Fig.1).

Treated group at day 7 of treatment with ASCs, revealed infiltration of polymorphic stem cells in the surrounding area of the renal arteriole, also there was an elongated fibroblast-like cells deposition in the necrotic renal tubules (Fig. 2) and in the peri-glomerular region (Fig. 3) as a replacement state.

Marked fibrin deposition in the peri-renal tubules area, and there was a marked area of fibroblasts proliferation in the renal parenchyma (Fig. 4).

![Figure (1): Control group at day 7 of treatment with normal saline infusion showed, hyperplasia of mesengial cells (A), as well to vacuolation of mesengial cells of glomerulus (B), in addition to necrosis of peri-](image-url)
glomerular renal tubules with infiltration of inflammatory cells (C). H&E. Stain. 40X

Figure (2): Histopathological section of kidney of treated group at day 7 of treatment with ASC₅ showed infiltration of polymorphic stem cells in the surrounding area of renal arteriole (A), also there are an elongated fibroblast like cells deposition in the necrotic renal tubules as a replacement state (B). H&E Stain 40X.

Figure (3): Histopathological section of kidney of treated group at day 7 of treatment with ASC₅ showed elongated fibroblast like cells deposition in the peri-glomerular region as a replacement state (A). H&E. Stain 40X.
Discussion

Artificial ischemic renal failure is induced by mechanical or medical method (4). In our research we used mechanical procedure and the induction time was 60 minutes. Monitoring serum BUN and creatinine levels alone are quite an accurate measure of renal function and a meaningful method in a clinical setting (17). Creatinine is a muscular metabolic product. As a more precise indicator of renal function than BUN, creatinine is a significant criterion of the severity of renal failure (18). Recently, the potential for renal repair by using stem cells has been clarified for various renal diseases. Among several sources of stem cells, adipose stem cells have attracted attention because of its ease of access (19). Effects of adipose stem cells on the damaged organs occur through mesenchymal stem cells fusion with existing host cells rather than via true differentiation (20). In our study, creatinine and blood urea nitrogen serum levels of the ASCs-treated ischemic group decreased after ASCs infusion compared with those observed in the ischemic renal failure injected with normal saline group this findings agreement with other workers (4). Treating animals with mesenchymal stem cells will result in a reduction of creatinine and urea plasma (8). MSCs may not only secrete cytokines within the injured kidney but also participate in endothelial cell proliferation or angiogenesis to facilitate renal regeneration (21).

To evaluate the therapeutic effects of ASCs in the ischemic renal failure group, we performed histopathological analysis which revealed infiltration of polymorphic stem cells in the surrounding area of the renal arteriole, also there was an elongated fibroblast-like cells deposition in the necrotic renal tubules and peri-glomerular region as a replacement state, in addition to marked fibrin deposition in the peri-renal tubules area, and there was a marked area of fibroblasts proliferation in the renal parenchyma, these finding reduce the damage in ASCs group. Many studies have revealed that after local damage to the tissue, MSCs replace their damaged counterparts in the bone, fat, liver, heart, brain, heart and skeletal muscle (22), MSCs therapy is effective, safe and reduced the rate of renal damage for patients with acute renal failure (20) and hastens the regenerative process (23).

The route of MSCs administration is more significant; in the other reports, intra carotid, jugular vein; cephalic vein, femoral artery and lateral caudal veins injection was used (22, 24, and 25). Many reports have shown that systematically administered MSCs move through the blood circulation and are ultimately retained at the liver, lungs or spleen (24, 26). Whereas we directly injected ASCs into target organ (kidney), as it could also
hold the cells. Migration of injected ASCs to damaged tissues can accelerate renal healing. Local vasculature procedure in cell therapy is an attractive approach in which select cells can be introduced into an aimed area and that will result in achieving cell localization with infusion little number of stem cells as well as it could minimize superfluous adverse effects and this considered an advantage in stem cells therapy as reported recently.

Conclusions

The administration of ASCs trans catheterization lead to improve renal excretory function, which was verified by the levels of serum BUN and creatinine in the canine ischemic renal failure model also play important role in renal tissue reconstitution there for, our findings suggest that ASCs could be an alternative therapeutic agent for canine ischemic renal failure.

Conflict of Interest: None

Funding: Self-funding

Ethical Clearance: The present study was approved by the Animal Welfare and Ethics Committee and Faculty of Veterinary Medicine/ University of Basrah.

Copy Enclosed

References


Correlation of PTEN Losses by Immunohistochemistry and Fish in Prostate Adenocarcinoma

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Abstract

Background: Prostate cancer (PC) is the largest and most unusual site of robust neoplasm in Europe, accounting for 22.8% of all newly diagnosed cancers in men. An increase in the prevalence of pentachloro anisole has been observed in most countries. This may be explained by the massive use of prostate unique antigen (PSA) in the early detection of PCa in the early stages. Objective: Detection of PTEN loss by IHC and FISH technique and compare between the two methods and correlate with Gleason grading system. Patients and method: A retrospective sample study was performed. From January 2014 - Joe. 2019. This study included 120 cases of prostate cancer from Baghdad and Duhok, with different age groups. Fixed formalin paraffin biopsy for prostatectomy in cases obtained from the Archives of Pathology Laboratories in Baghdad” (Shahid Ghazi Hariri Training Hospital and Laboratories). 

Results: PTEN (FISH) detected the gene in 29 case and confirmed by PTEN (IHC) expression results, underestimation occurred in five case detected by PTEN (IHC). Concerning PTEN (IHC), it detected the gene in 34 case. Percentage of cases showing negative staining for PTEN protein in high grade was higher than cases showing positive protein expression (good correlation with new grading group). IHC to detect PTEN protein levels is an interesting alternative to knowledge of the FISH PTEN gene loss and as a prognostic biomarker. There was significant association between PTEN gene expression and age. PTEN (IHC) showed negative results in 65.3% of case; while loss was shown in 58.2% of s by PTEN (FISH). 

Conclusion: The important result in this study was a substantial agreement between the expression results of PTEN (FISH) and PTEN (IHC). 

Keyword: PTEN (FISH), PTEN (IHC), Prostate adenocarcinoma, immunohistochemistry

Introduction

Prostate cancer (PCa) is the largest and most unusual site of solid neoplasm in Europe, accounting for 22.8% of all newly diagnosed cancers in men. An increase in the prevalence of PCa has been observed in most countries. This may be explained by the massive use of prostate unique antigen (PSA) for detection of PCa in early stages. In Iraq, prostate cancers represent the seventh most common malignancy in Iraq Registry for 2012. 

(4) Most prostate cancers are detected by virtual rectal exam (DRE), and transurethral ultrasound, however, ultrasound misses almost 30% of isoechoic carcinomas, or PSA improvement (both above 4ng / dL and growing over time). Nowadays, needle biopsy identifies several prostate cancers. Less often – its miles recognized by transurethral resection samples. Prostate cancers can be classified into:

(1) Adenocarcinomas (“secondary”) of peripheral ducts and acini ,

(2) large (“primary”) ductal carcinomas.

Roughly speaking, a lump can be hard to look, but it can usually be diagnosed as a poorly defined gray or
yellow area, poorly delineated or corporation area.

Early detection tasks can detect minor tumors. (5)

**Microscopically:** The four basic structures of cellular architecture patterns are the medium sized glands, little glands, diffuse single infiltration of cells and the cribriform form.

It is accompanied by a cytological incongruity in the nuclear enlargement, contour irregularity, hyperpigmentation and most importantly notable nucleoli (“macro-nucleoli” ,Calibration > 1 μm in diameter). Nucleoli are typically numerous and more likely to be emarginated. Mitosis also important, however, it is rare in a well-differentiated tumor. (5)

The types of prostate cancer that form glands are usually lined with a single cell layer, but rarely contain stratified epithelial tissue that mimics PIN (6) and have many benign mimics (7)

**Materials and Methods**

During the period from January, 2014 to June 2019, a retrospective selective study was performed included 120 cases of prostate cancer with different age groups from Baghdad and Duhok.

Fixed formalin paraffin biopsy for prostatectomy obtained from the Archives of Pathology Laboratories in Baghdad” (Shahid Ghazi Hariri Teaching Hospital and Laboratories, Duhok Central Health laboratory and Vajeen specialist laboratory & certain private clinical laboratories.

Additionally, normal prostate in the form of paraffin blocks is a positive control (PTEN), and kidneys with RCC is a positive control (AMACR).

Patients’ clinical data, including age and provisional clinical diagnosis, were obtained from archival histological reports.

Each H&E stained slide was reviewed for pathological evaluation and diagnostic evaluation to confirm the diagnosis of prostate cancer. Cases are scored and graded in accordance with new grading.

Immunohistochemical technique and molecular work of the study were performed in Duhok (Vajeen specialist laboratory).

**Inclusion Criteria**

Various types of prostate specimens were included in this study, including transurethral resection of the prostate, needle biopsy, and resection of the prostate.

**Exclusion criteria:**

Insufficient biopsies, poorly preserved prostate specimens, and biopsies with acute inflammation and necrosis were excluded.

Tissue Microarray Technology (TMA):

A new technique used in this study which is Tissue-Microarray

The procedure involves using a hollow needle to cut core of tissue just 2 mm in diameter from the areas of interest (previously detected prostate tumor areas in stained glass (H&E slides) into paraffin embedded tissue.

Sections of the Microarray blocks are excised by a microtome, equine on a single microscope slide, then evaluated by staining with H and E, then another 4 μm section is made for the immunohisto-chemical stain for PTEN and one section for the FISH probe.

**Immunohistochemical technique:** Agilent Company ®.

A. The following equipment was used during the study For IHC:

- Microtome
- Water bath
- Glass slides.
- Microwave oven.
- Humidity champer
- Optical microscope.
- FLEX IHC microscope slides (code K8020)
- Silanized chip (code S3003)
- Buffer solution - phosphate saline (PBS).
xylene.  
- Envision™ FLEX Hematoxylin (counter stain) Link (code K8008).  
- Distilled water.  
- Ethanol  
- Aqueous mounting medium  
- Permanent mounting medium  
- Dako Autostainer / Autostainer Plus and Dako PT Link Tools.  

B. PTEN Primary Antibody:  
- monoclonal mouse against human clone PTEN 6H2.1, catalog number M3627; Made in Dako, USA.

**Immunohistochemical method:**

Immunohistochemical work in this study was performed with an automatic Link 48 autostainer. Micron sections were obtained from formalin-fixed and paraffin-embedded tissue blocks, a section utilizing Dako PT 3-in-1 specimen preparation procedure. Follow the pre-treatment procedures posted in the EnVisionTM FLEX Target retrieval Solutions. Height of PH (10x) and PH 9 (code S2388) for PTEN.

Post staining the sections, they were dried, cleaned and impregnated with a permanent fixing medium.

**Fluorescence in Situ (FISH) technique:**

A. Equipment and materials required to study FISH in this research:

Zytolight FISH Tissue Incorporation Kit (serial number Z-2028-5 / -20.  
Positive and negative control specimens  
Positively charged slides  
Water bath (37 °C - 98 °C)  
Hybridization / hotplate  
Hybridization or humidity chamber in the hybridization oven

- Adjustable pipettes (10 ml, 25 ml)  
- Staining: cans or baths  
- Timers  
- Calibrated thermometer  
- Xylene  
- Distilled or Deionized water  
- Cover-slips (22 mm x 24 mm x 60 mm)  
- Rubber glue, such as B. fixogum cement-based rubber.  
- Fluorescence microscope (400-1000X)  
- Certified immersion oil for fluorescence microscopy  
- Appropriate filter sets

**Reagent provided:**

The Zytolight SPEC PTEN / CEN 10 dial probe consists of:

ZyGreen-labeled polynucleotide (excitation 503 nm / irradiance 528) (10 ng / mL, target sequence mapping in 10q23.2-q23.31 * (chr 10: 89,440,649-89,755,790) contains a region of PTEN gene

ZyOrang-labeled (547 nm excitation / 572 nm irradiance) polynucleotide (1.5 ng / mL) targeting the sequences shown in the 10p11.1-q11.1 of the alpha satellite central region of the D10Z1 chromosome 10.

Hybridized Buffer based on formameda.

**Counter –stain vial:**300 μl per vial (30 tests). The counterstain is DAPI antifade (ES: 0.125 μg / ml DAPI (4,6-Diamidino-2-phenylindole)).

Cytocell comp. manufactured a pre-treatment kit and a removal kit.

B. Fluorescence in the in situ hybridization method:

Preparation of solutions used in FISH workings:

Sodium chloride sodium citrate (SSC solutions):
- Sample preparation:
  - A section of tissue placed on a charged slide, then preserved in the oven at 55 °C overnight to remove paraffin
  - Then dehydrated in the next day

Pretreatment of tissue samples:

It consists of two stages:

1. Heat pre-treatment
2. Enzymes-Digestion

The samples then dried at room temperature in a 75%, 85% and 100% series of ethanol for 2 minutes at each concentration. Keep the slide for air drying and proceed to predenaturation, denaturation and hybridization.

FISH signaling analysis:

A fluorescent microscope should be available. Use the DAPI / FITC / Texas Red triple-band filter for optimal green, red, and DAPI fluorescent imaging.

Normal state: In interphases of normal cells or cells while not a deletion involving the PTEN gene region, two green and 2 orange signals appear.

Aberrant situation: In a cell with deletion affecting the PTEN gene region, a reduced number of green signals will be detected. Deletion affecting only parts of the PTEN gene region might result in normal signal pattern with green signals of reduced size

statistical analysis:

All statistical procedures, data management and analyses were performed with the aid of the SPSS,IBM,US, version 25, The Kappa Cohen value was used to assess the degree of consistency and agreement of PTEN expression results FISH and PTEN(IHC). All analyses done under the assumption that p. value was two tailed and ≤ 0.05 to be considered significant

Results

A total of cases 98 cases with proved diagnosed adenocarcinomas were enrolled in this study.

Age Distribution

As it shown in figure 1, age distribution of the cases revealed that a mean age of the studied group was 70.15 ± 8.8 (range: 50-95) years. Higher proportion of cases aged 70 years or older accounted for (60.2%) of cases.

Figure 1: Age distribution of the studied group

PTEN (IHC and FISH)

Results of PTEN test using immunohistochemistry and FISH are shown in table 1. PTEN (IHC) showed negative results in 65.3% of cases; while the loss was detected at 58.2% by PTEN (FISH).

<table>
<thead>
<tr>
<th>table 1: PTEN results by immunohistochemistry and FISH</th>
<th>a variable</th>
<th>No (n = 98)</th>
<th>percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN (IHC)</td>
<td>negative</td>
<td>64</td>
<td>65.3</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>34</td>
<td>34.7</td>
</tr>
<tr>
<td>PTEN (FISH)</td>
<td>Loss</td>
<td>57</td>
<td>58.2</td>
</tr>
<tr>
<td></td>
<td>No losses</td>
<td>41</td>
<td>41.8</td>
</tr>
</tbody>
</table>
Figure 2  negative staining PTEN immunohistochemistry  prostatic adenocarcinoma score 9 (4+5) Grade 5

Figure 3  negative staining PTEN immunohistochemistry with internal positive control of PTEN benign prostatic hyperplasia
PTEN (FISH) detected the gene in 29 cases and confirmed by PTEN (IHC) expression results, underestimation occurred in five cases detected by PTEN (IHC). Concerning PTEN (IHC), it detected the gene in 34 cases. Percentage of cases showing negative staining for PTEN protein in high grade was higher than cases showing positive protein expression (good correlation with new grading group). IHC to detect PTEN protein levels is an interesting alternative to knowledge of the FISH PTEN gene loss and as a prognostic biomarker. There was significant association between PTEN gene expression and age.

Relationship between PTEN expression (IHC) and PTEN gene expression by (FISH).

PTEN (FISH) detected the gene in 29 cases and was confirmed by the results of PTEN (IHC) expression. Underestimation occurred in five cases (detected by PTEN (IHC). As for PTEN (IHC), the gene was detected in 34 cases.

During tissue sections, a part of the cell and the nucleus can be cut, resulting in a “truncation effect” in which loss of the PTEN signal from the nucleus can be erroneously estimated as deletion of the gene. Therefore, it is very important that false positive results, which were probably the result of truncation, were determined by comparison with normal nuclei in all FISH deletion tests. (8)

Another explanation for the false negative result is a technical error in the FISH procedure due to air bubbles while the cap is slipping. This prevents the probe from reaching the desired area.

Twelve cases showed presence of a gene by FISH procedure, which is not confirmed by immunochemotherapy, in fact there were two explanations for this finding, the first cause may be exposure to microRNA (miR-4534 directly represses the tumor inhibitor PTEN gene). The second is due to a point mutation in 10q23.3. (9).

An important outcome in this study was the significant substantial agreement between PTEN (FISH) and PTEN (IHC) expression results, and this agreement was statistically significant (kappa = 0.635, P = 0.001).

The PTEN (IHC) sensitivity was 70.7%, specificity 91.2%, and accuracy 82.7%.

In the same direction with this study, another study found that low PTEN expression was significantly associated with PTEN genomic removal which indicated that erasure is the main procedure erasures as the main method causing reduced PTEN protein expression.

In 28/39 cases (72%), a similarity was observed between deletion state of PTEN and lower expression of PTEN protein. Moreover, in eight cases out of eleven disagreement cases, grade I (declined) or grade
0 (absent) expression of PTEN protein were detected, these 8 cases were negative for PTEN deletion. While normal expression of PTEN was reported in the other 3 cases. (10)

The possibility that PTEN FISH might not recognize some cases of prostate cancer with PTEN inactivated greatly supports the need for replacement analysis to characterize PTEN loss.

IHC for detection of PTEN protein levels is a clear alternative for detecting PTEN FISH gene loss. Four previous studies assessed the usefulness of PTEN-IHC in prostate cancer as predictor of loss of PTEN. (11-14)

some of which are currently in clinical trials for prostate cancer. PTEN loss could also be a biomarker of resistance to hormone therapy in advanced prostate cancer. (15) The opportunity that PTEN FISH may fail to distinguish some cases of cancer of the prostate with PTEN deactivation powerfully maintains for the need for a

The PTEN protein expression by IHC is a simple analysis that is easier and cheaper to perform than FISH. As it is compared to FISH, this test is easier to be performed by needle biopsy-sampling and may indicates PTEN protein absence among additional cases. Therefore, when there PTEN protein is lack or absent on needle-biopsy specimens it could be a promising predictive biomarker that is useful in screening patients with low risk prostate cancer who are likely to progress, where there will be a need to treatment and intervention. (15)

Moreover, loss of PTEN as a biopredictive marker may help select appropriate patients for treatment with Novel-PI 3-kinase pathway therapies, nonetheless, currently these therapies are under investigations by different clinical trials for treatment of prostate cancer. From other point of view, in advanced cases of prostate cancer, loss of PTEN can be used as an indicator-biomarker to predict resistance to hormonal therapy. (16)

Conclusion

The important result in this study was a substantial agreement between the expression results of PTEN (FISH) and PTEN (IHC).


Impact of Diastasis Recti Abdominis and Low Back Pain on Quality of Life in Post-Partum Female

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Abstract

Introduction: The separation of the two rectus abdominis muscle along with linea alba is called diastasis recti abdominis. Diastasis recti is common in Post-partum females. In Post-partum period Low back Pain is very common which lead to daily activity limitation. Post-Partum women complaint regarding there usual activities, mobility, pain, which affect the quality of life in individual.

Aim: To study the” Impact of diastasis recti and low back pain on quality of life in post-partum females”

Method: This study will be carried out in Physiotherapy OPD, Ravi Nair Physiotherapy College and AVBRH, Sawangi (Meghe), Wardha. Diastasis recti will be evaluated in Post-partum females. Impact of Diastasis recti and Low back pain on Quality of Life in post-partum female. Low back pain is going to asses with the help of Modified Owestry low back pain disability questionnaire .And quality of Life with the help of Maternal postpartum questionnaire

Results: Study yet to be commenced.

Conclusion: Conclusion will be published after the result are analysed.

Keywords: Diastasis recti, Post-Partum female, Low back Pain, Quality Of Life.

Introduction

Diastasis recti abdominis is defined as the separation of the two rectus Abdominis muscle along the linea alba¹. Mechanical effect of pregnancy on the Abdominal musculature is mediated hormonally in diastasis rectus abdominis¹. Diastasis recti abdominis occur due to weakening of anterior abdominal wall due to mechanical strain¹. The space form in diastasis recti abdominis due to Separation of linea alba is Inter-recti abdominis¹. Measurement of diastasis recti done with the help of dial caliper⁸. In postpartum period diastasis recti Abdominis is more common¹. 4th Stage of Labor is sometimes referred to as postpartum⁵. Post-partum extend up to 6 month after giving birth⁵. Risk factor mainly occur in post-partum women generally include physical health risk⁵. Diastasis recti is seen 68% immediately after post-partum³. At the 8 week of Post-partum the inter-recti distance resolve gradually with the time and with Individual validity¹. At 12-14 week of post-partum and 6 month of postpartum mota et al asses inter-recti abdominis¹. The universal health problem for all age group is low back pain, of these Postpartum low back pain is the more common and which lead to daily activity limitation⁶. More than 6 month of postpartum more prone to diastasis recti. It may change posture and give more back strain, which lead to reduce strength and function that lead to occur of low back pain⁷. With in half a year after delivery 4 out of 10 women report of persistent low back pain and 20% of women with back pain report persistent symptom

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DOI Number: 10.37506/ijfmt.v15i1.13528
at the 3 year of postpartum. Lumbopelvic pain occur in pregnant women which may persist or arise after delivery. Women with diastasis recti often experienced back pain. To provide the proper support to the trunk and spine our body require strong and intact abdominal muscles. During activity more demand gets place on the spine because of improper support from the abdominal muscle so pain begin at the spine. Post-partum women report complaint regarding mobility, Self-care, usual Activities, pain or discomfort which affect quality of life in individual. At the end of 1st month there is limited problem in the women and can perform usual activities.

Rationale:
Various studies have been undertaken which concluded that diastasis recti is one of the cause for prevalence of low back pain in postpartum women even after 1 year of delivery resulting in persistence of symptom of low back pain that affecting the functional activity of female which can hamper quality of life therefore need was felt to carry out the current study to find the effect of Diastasis Recti on Quality of Life in Postpartum women with Low back pain.

Objectives:
1. To Evaluate of width of diastasis recti
2. To Evaluate of low back pain
3. To Assess quality of life using
4. To find association of diastasis recti with low back pain
5. To correlate diastasis recti with QOL.
6. To correlate low back pain with quality of life.

Methods
Study design: Observational study

Study setting: AVBRH Hospital and Physiotherapy OPD.

Participants:

Inclusion Criteria:
1. Females with diastasis recti
2. Upto 6 months post-partum females
3. Post – partum Females having low back pain
4. Age 20 to 35 years

Exclusion Criteria:
1. Females with lower limb radiculopathy
2. Females with PIVD or Stenosis

Variable:
1. Width of diastasis recti
2. Severity of Low back pain
3. Quality of life score

DATA SOURCE / MEASUREMENT:

For diastasis recti – width of gap between the two bellies of Rectus Abdominis will be measured using digital caliper

For severity of Low back pain – score of Modified Oswestry low back pain disability questionnaire will be measured

For Quality of life – Score of Maternal postpartum questionnaire will be measured

Bias: Subjects not fulfilling the selection criteria will be excluded from the study to prevent bias

Study size: 175

Statistical method: Simple random sampling

Result: Upon completion of the study results, statistical analysis will be estimated.

Discussion

The current study is carried out to find the impact of diastasis recti and low back pain on Quality of life in postpartum females. Various studies carried out have
reported back pain can affect the quality of life. The weak abdominal result in imbalance of muscle support to the truck and can over stress the back muscle causing back pain, in postpartum females due to pregnancy the abdominal muscles are stretched causing weakness and even separation of rectus muscle if this persists support to the trunk may be compromised causing back pain and this may prevent female from carrying out various activities even taking care of her newborn and may affect her Quality of life. Thus, current study aims to find out effect of diastasis recti and low back pain on quality of life in postpartum females.

**Key results:** Diastasis recti, Post-Partum female, Low back Pain, Quality Of Life.

**Limitation:** It might be difficult to convince patient for being a part of this study.

**Generalizability:** Study not done yet.

**Conflict of Interest:** There is no conflict of interest.

**Source of funding:** No funding needed

**Ethical Clearance:** Being an observational study institutional ethical committee permission was taken.

**References**


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The Influence of Interpersonal Communication on the Prevention Action of Dengue Hemorrhagic Fever (DHF) in Meo-Meo Public Health Center of Baubau City

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Abstract

Dengue infection is a global health problem. One of the city in Indonesia that has a high incident of DHF is Baubau City, especially the area of Meo-Meo Public Health Center in 2016 to 2018 ranked first of the incident of DHF respectively for three years among 10 Public Health Center on the coastal area and third among 17 Public Health Center in Baubau City. DHF cases are not only caused by mosquitoes, but also by human behavior that does not carry out healthy and indifferent lifestyles in the environment where mosquitoes nest. Such behavior includes leaving the hanging used clothes, does not drain the tub, leaving puddle around the residence. This study involved 39 people in the intervention group and also in the control group who met the criteria including the age of 17-65 years old, can read, write, and communicate well, and willing to participate in this study. The result indicates a significant from the intervention groups to the control group (p< 0.05), is action (p=0.001), and the difference in the percentage of action in both groups. So, it can concluded that interpersonal communication is more influential than counseling on increasing DHF prevention action.

Keywords: interpersonal communication, counseling, action, prevention, Dengue Hemorrhagic Fever (DHF)

Introduction

According to WHO, dengue infection is a global health problem with an estimated incidence of around 390 million people each year. Asia is headed for the dengue epidemic in 2019. Several countries including Australia, Cambodia, Laos, Malaysia, the Philippines, Singapore, and Vietnam have been facing a surge in dengue cases in the past six months1. DHF cases until the beginning of February 2019 reached 16,692 cases with 169 people dead. This number increased compared to the previous month, which was 13,683 cases with 133 people died. This number increased compared to the previous month, which was 13,683 cases with 133 people died. Most cases of DHF in Indonesia are in the regions of East Java, Central Java, NTT and Kupang2.

Cases of dengue in Southeast Sulawesi in 2018 amounted to 624 cases, with the number of deaths of 4 people. This figure decreased from 2017, which was 817 cases, and while the number of deaths was as many as 12 people. DHF Incidence Rate (IR) in 2018 decreased compared to 2017, which is from 31.39 to 23.51 per 100,000 population. The increase in case fatality rate (CFR) from the previous year was 0.26% in 2017, to 0.64% in 20183. In Baubau City, in 2018 the number of DHF patients was 98 which was decreased from 116 cases in 2017, but increased again to 157 cases in 20194.

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DHF cases in the area of the Meo-Meo Public Health Center in Batupoaro Sub-District, Baubau City were 11 in 2015, 20 in 2016, 17 in 2017, 10 in 2018, and 10 in 2019. Meo-Meo Public Health Center in 2016 – 2018 ranked first in the incidence of DHF cases respectively for three years among 10 Public Health Center on the the coastal area and third among 17 Public Health Center in Baubau City. In 2019 there were still 10 cases of DHF.

DHF cases are not only caused by mosquitoes, but also by human behavior that does not carry out healthy and indifferent lifestyles in the environment where mosquitoes nest. Such behavior as includes leaving the hanging used clothes, does not drain the tub, leaving puddle around the residence.

Shidiq’s research found that counseling was effective against increasing knowledge, attitudes, and actions but not effective against the existence of larvae. Research by Ikhlasari showed that there was a significant relationship between education, income, and knowledge in the control group. There is a relationship between the control group and the treatment group on the aspects of knowledge and attitudes that indicated that the administration of DHF prevention interventions is related to knowledge and attitude.

Hutapea’s research stated that interpersonal communication conducted by parents was seen as effective and successful in preventing dependence on others in the feelings and abuse of drugs in adolescents. Interpersonal communication is communication between a communicator and a communicant. Interpersonal communication is also known as the communication of people by face-to-face that allows each participant to capture the reactions of others directly, both verbally and nonverbally.

Interpersonal communication is verbal and non-verbal interaction between two or sometimes more than two people who are interdependent with each other. Interpersonal communication aims to learn, build relationships with others, influence others, and help others. Interpersonal communication is carried out through face to face where the source of the message can see who the recipient of the message is. Then, there is immediate feedback without having to use an intermediary. Therefore, communication participants can also easily and immediately receive feedback from other communication participants at that time.

To overcome the DHF cases, various efforts have been carried out including larvae monitoring by the DHF Team of Baubau City Health Office where the target is the Public Health Center area where DHF cases are found, cross-program and cross-sector meetings, fogging in areas where DHF cases occur, giving abate powder by Public Health Center staff in each of the houses of the Health Center working area, counseling the Mosquito Eradication Movement (PSN) movement in all walks of life.

The health promotion efforts that have been done were not able to cultivate the community partisipan in PSN. The effort to break the chain of infection of dengue fever with the PSN movement will not be meaningful without the awareness of the community itself. So the purpose of this study was to determine the effect of interpersonal communication on DHF prevention measures.

**Material and Method**

This research was an experimental study through Quasi-Experiment with a non-randomized pre-test – post-test control group design. The intervention group and the control group presented a pretest to find out the primary state then the intervention group was given interpersonal communication and the control group was did counseling. After the treatment, both groups performed a post-test with the same questionnaire. The distance between the pre-test and post-test for approximately 2-3 weeks or a maximum of 4-5 weeks.

The following is the method scheme:

<table>
<thead>
<tr>
<th>Pretest</th>
<th>Posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>P</td>
</tr>
<tr>
<td>O3</td>
<td>P1</td>
</tr>
</tbody>
</table>

O₁ Pre-test for intervention group related to knowledge, attitude, and action on the prevention of DHF before given interpersonal communication

O₂ Post-test for intervention group related to knowledge, attitude, and action on the prevention of DHF after given interpersonal communication
O3 Pre-test for control group related to knowledge, attitude, and action on the prevention of DHF before given counseling

O4 Post-test Pretest for intervention group related to knowledge, attitude, and action on the prevention of DHF after given interpersonal communication

P The intervention using interpersonal communication

P1 The intervention using counseling

Based on the calculation of the minimum sample and those who met the inclusion criteria including eth age of 17-65 years old, can read, write, and communicate well, and willing to participate in this study, 39 households for the intervention group and 39 households for the control group were chosen.

Result

Table 1: The Pre-test and Post-test Percentage Score of Action in the Interpersonal Communication Group and Control Group on the Prevention of DHF

<table>
<thead>
<tr>
<th>Action</th>
<th>Interpersonal Communication (%)</th>
<th>Counseling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor (0-5)</td>
<td>16 (41)</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Fair (6-7)</td>
<td>5 (13)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Good (8-10)</td>
<td>18 (46)</td>
<td>31 (79)</td>
</tr>
<tr>
<td>Rerata</td>
<td>7 (Fair)</td>
<td>9 (Good)</td>
</tr>
</tbody>
</table>

| Post-test    |                                 |                |
| Poor (0-5)   | 0 (0)                           | 0 (0)          |
| Fair (6-7)   | 0 (0)                           | 0 (10)         |
| Good (8-10)  | 39 (100)                        | 39 (100)       |
| Average      | 10 (Good)                       | 9.85 (Good)    |

Table 1 shows the percentage of scores of action before and after interpersonal communication intervention increased significantly, especially in the good category where at pre-test showed a rate of 46% and improved after the intervention with a percentage of 100% with an increase in the mean value from 7 (fair) to 10 (good). Similarly happened in, the control group in which, at the time of achievement, the percentage of the good category was 79% and increased after the intervention to 100% with the average pre-test score of 9 (good) and the average post-test score increased to 10 with a good category.
Table 2: Score of Action in the Interpersonal Communication Group and the Control Group on Prevention of DHF

<table>
<thead>
<tr>
<th>Group</th>
<th>Score of Action</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Average</td>
</tr>
<tr>
<td>The Intervention Group</td>
<td>39</td>
<td>6.77</td>
</tr>
<tr>
<td>Pre-test</td>
<td>39</td>
<td>10.00</td>
</tr>
<tr>
<td>Post-test</td>
<td>39</td>
<td>10.00</td>
</tr>
<tr>
<td>The Control Group</td>
<td>39</td>
<td>8.56</td>
</tr>
<tr>
<td>Pre-test</td>
<td>39</td>
<td>9.85</td>
</tr>
<tr>
<td>Post-test</td>
<td>39</td>
<td>9.85</td>
</tr>
</tbody>
</table>

Table 2 shows that the mean score of pre-test (6.77) and post-test (10.00) increased in the intervention group. The statistical test results obtained $p = 0.000$ ($p < 0.05$) shows that there were significant differences in the mean score of the pre-test and post-test scores on the increase in prevention of DHF.

The increase in the mean score of actions also occurred in the control group at pre-test (8.56) and post-test (9.85). Statistical test results obtained $p-value = 0.000$ ($p < 0.05$) showing that there was a significant difference in the score of the pre-test and post-test actions to increase the prevention of DHF.

Table 3: The Difference Score of Actions in the Interpersonal Communication Group and Control Group on the Prevention of DHF

<table>
<thead>
<tr>
<th>Action</th>
<th>Statistical Score</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Average</td>
</tr>
<tr>
<td>Pre-test</td>
<td>39</td>
<td>7.67</td>
</tr>
<tr>
<td>Intervention</td>
<td>39</td>
<td>1.50</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>1.50</td>
</tr>
<tr>
<td>Post-test</td>
<td>39</td>
<td>7.92</td>
</tr>
<tr>
<td>Intervention</td>
<td>39</td>
<td>1.50</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Table 3 shows the results of the statistical test on the pre-test score of the action between the intervention group and the control group which was not significant, with the value of $p = 0.079$ ($p < 0.05$). Furthermore, the results of significant in which, the value of $p = 0.001$ ($p > 0.05$). The post-test score of the intervention group was 7.67 which was higher than the control group of 1.50, in which the difference score is 6.17.

**Discussion**

The selection of research sites was based on the consideration that Lanto Village had the highest cases of
Dengue Hemorrhagic Fever (DHF) for the last 3 (three) years. Likewise, Wameo Village had the lowest cases of DHF for 3 (three) years. Most recently in the area of Meo-Meo Public Health Center. This was based on the results of an initial survey through interviews with DBD programmers of Baubau City Health Office and the health profile data of Baubau City in 2019.

Statistical test results in the interpersonal communication intervention group obtained $p = 0.000$ ($p < 0.05$) showing that there were significant differences in the scores of pretest and posttest actions to increase the prevention of DHF prevention measures. The results of the statistical test in the counseling control group obtained a value of $p = 0.000$ ($p < 0.05$) showing that there were significant differences in the mean score of the pretest and posttest scores on the increase in the prevention of DHF.

Based on the statistical tests of the two groups, both are significant. However, there was a difference in the percentage of the attitude of the intervention group which was 47.7% higher than the control group of 15% with a difference of 35.3%, as well as a higher score in the intervention group by 0.15. So, it can be concluded that interpersonal communication was more influential than counseling on increasing the prevention of DHF.

This is in line with research conducted by Aysha et al, that there was a relationship between the eradication of mosquito breeding and DBD events. Furthermore, research by Ayudyah et al shows a relationship between knowledge, attitudes and preventive measures for DHF disease vectors. The prevention action of DHF can be done with several appropriate methods, one of which is controlling the mosquito vector by draining the bathtub, burying used cans, etc.

The results of this study are also in line with research carried out by Weningtyas et al. that interpersonal communication influenced customer satisfaction and service quality to customer satisfaction. Research by Patriana also showed that interpersonal communication between social counselors and the families of criminal offenders runs effectively because it fulfilled elements such as trust, openess, mutual support, and empathy.

This is reinforced by the Hutapea’s study that interpersonal communication was most effectively used by parents in preventing drug abuse in adolescents. Maharja also stated that good interactions tend to lead to good behaviors.

Interpersonal communication is communication carried out in an interpersonal relationship between two or more people, both verbally and nonverbally, to achieve the same meaning of communicating with others in the context of interpersonal communication, it is not only about communicate verbally, but also nonverbally. Nonverbal communication in the interpersonal communication process serves to complement verbal communication.

Giving interventions in the form of interpersonal communication present to the intervention group and counseling to the control group facilitates the delivery of information so that both groups can receive the message delivered and directly influence the pretest and posttest scores in both groups.

**Conclusion**

Based on research, it can concluded that there is an increase in actions before and after counseling on the prevention of DHF in the area of Meo-Meo Public Health Center in Baubau City. Furthermore, it finds that interpersonal communication is more influential than counseling on increasing DHF prevention behavior in the working area of Meo-Meo Public Health Center in Baubau City.

**Conflict of Interest:** None

**Source of Funding:** Self

**Ethical Clearance:** Health Research Ethics Committee, Faculty of Public Health, Universitas Hasanuddin

**References**


The Coronal Pulp Cavity Index an aid in age determination - A Cone Beam Computed Tomography Study

Ceena Denny E1, Bastian TS2, Srikant Natarajan3, Nithin Thilak4, Almas Binnal5
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Abstract

Background: The objective of the present study was to assess the accuracy of age estimation from TCI of mandibular molars (except wisdom teeth) of both sides using CBCT images of known age and sex, so that we can apply this method to estimate age in both living individuals and skeletal material of unknown age.

Methods: A cross sectional retrospective study was conducted on a total of 100 CBCT images of subjects who were referred to the department for varied diagnostic purposes of known age and gender. All CBCT images with a fully visible pulp cavity were selected. Two measurements were taken at two different levels. Height of the crown (CH) and height of the coronal pulp cavity (CPCH). Then tooth – coronal index (TCI) for each tooth was calculated as follows: TCI = CPCH X 100 / CH.

Results: Comparison of TCI value between male and female showed no significant difference between the gender. Among the four mandibular molars (lower left and right 1st and 2nd molars) 46 and 47 TCI correlates well with age. TCI of 46 has significant prediction of age in female with an R value of 0.426. Inter observer measurements showed a moderate to good agreement of the measures.

Conclusions: TCI method of mandibular teeth was found to be a reliable method for age estimation and not gender determination. CBCT can be used in age determination for forensic purposes as it is non-invasive and also makes it possible to reconstruct the images in different planes showing the anatomical and imaged structures at different planes.

Key words: Coronal Pulp Cavity Index, Age Estimation, Cone Beam Computed Tomography, Forensic Odontology.

Introduction

Saunders, in 1837 was the first to publish an article ‘Teeth A Test of Age’ implying the importance of teeth in age estimation. In forensic dentistry, age determination using teeth plays a vital role in identification of the victim during mass disaster, criminal cases or social issues. Teeth being the hardest structure also is least resistant to decomposition. Various age estimation methods require extracted tooth by sectioning which is not feasible in a living individual.

The size of the canal and the pulp chamber is inversely proportional to human age. The various age related changes seen associated are odontoblastic vacuolization, reticular atrophy, fibrosis of pulp, hyaline and mucoid degeneration and diffuse calcification. Reparative dentine formation also results in decrease of the pulpal volume due to wasting disease, trauma and restoration.

Age estimation using Tooth coronal index (TCI) has been done using 2 dimensional (2D) radiographs like intra oral periapical (IOPA) using the paralleling technique and orthopantomograph (OPG). It was found to be simple and cost effective than histological methods and could be used in both living and un identified dead for age estimation. Even though, conventional radiographs have been widely as a non-destructive method in the measurement of the pulp chamber. The
disadvantages of these 2D radiographs is that it could have projection errors and the tooth could not be assessed in all directions from a single radiograph. Cone Beam Computed Tomography (CBCT) scans has overcome these disadvantages of 2D imaging modalities as it is also non-invasive. A 3 dimensional (3D) reconstructed image can be obtained which allows us to visualise the morphology of a tooth from all angles without any image distortion.

The present study was done to assess the accuracy of age estimation from TCI of mandibular molars (except wisdom teeth) of both sides using CBCT images of known age and sex, so that we can apply this method to estimate age in both living individuals and skeletal material of unknown age.

**Method**

A cross sectional retrospective study was conducted on a total of 100 CBCT images of subjects who were referred to the department of Oral Medicine and Radiology for varied diagnostic purposes of known age and gender. The study protocol was approved by the Institutional Ethics committee. The inclusion criteria for selecting the images were those images of patients with healthy teeth without any periapical or periodontal pathologies. The second requirement was that the scanned images were of good diagnostic quality without any artefacts. The images excluded were of those patients with history of trauma or pathology to the teeth and in which the pulp that could not be identified. Images of patients with syndromes or any congenital disorders were excluded. The study images were taken using Promax 3D, Mid version (Planmeca Oy., Helsinki, Finland) CBCT unit. CBCT images were chosen over panoramic images as the measurements were more accurate in terms of magnification and better individual detail could be obtained as there were no superimposition of other structures.

The study images were of 200 subjects (56 females and 44 males). Four mandibular molars (lower left and right, 1st and 2nd molars) excluding the 3rd molars were assessed. The individual tooth was assessed in the axial section and was aligned in the coronal section so that the long axis of the tooth was perpendicular to the lower border of mandible. The measurements were taken in the sagittal section after proper alignment in the axial and coronal section. All CBCT images which with a fully visible pulp cavity were selected. Two measurements were taken at two different levels. Height of the crown (CH) and height of the coronal pulp cavity (CPCH). Then tooth – coronal index (TCI) for each tooth was calculated as follows: TCI = CPCH X 100 / CH.

All measurements were taken using the same machine. To ensure the accuracy of the technique used for measuring TCI detailed reference points were used: Cervical line that connect two landmarks to be measured; the mesial and distal cemento-enamel junction points; and divides the tooth into crown and root. Crown height is the maximum perpendicular distance from the cervical line to the tip of the highest cusp of teeth. While pulp height is the distance from cervical line to the coronal tip of the pulp chamber as shown in Figure 1.

After the procedures of image acquisition and measurement of the height of the crown and the pulp cavity as described above, the volume of pulp chamber was calculated using the region growing tool using the Romexis software as shown in Figure 2.

The measurements were taken using the software–based calibrated measurement tool. All measurements were carried out twice by two observers and the mean was recorded to minimize intra and inter-observer errors.

**Statistical Analysis**

The statistical analysis of data was done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 20. Independent t-test was used for comparison of gender. Linear regression analysis was used for prediction of age using TCI of 36, 37, 46 and 47. Interclass correlation coefficient for interobserver variability between 2 observers for the calculated parameters of TCI.

**Results**

Interobserver variability was carried out using Interclass correlation coefficient test. The ICC values were 0.730, 0.592, 0.679 for male, female and total population respectively for measurement of TCI (P value of <0.001) which indicated moderate to good agreement of measures.
Independent students t test was used to compare TCI value between male and female and showed no significant difference between the gender (Table 1).

Linear regression analysis was performed to predict the age of the individual using the TCI of the first and second molars. Significant association was seen with the TCI values of the tooth 46. Among the four molar teeth 46 and 47 TCI correlates well with age (AGE =13.808+0.131(TCI 46), SEE of 4.25 years and AGE =14.912+0.124(TCI 47), SEE of 4.374 years). TCI of 46 has significant prediction of age in female with an R value of 0.426 and standard estimate of error (SEE) of 3.71 years (AGE =13.651+0.14(TCI 46)). This was the best predictor equation. The other two significant equations were total population equation of 46 and 47. TCI values, with an R value of 0.333 and 0.241 respectively.

Independent t test was done to compare the volume of the pulp among both the sexes and it showed a statistical significant difference between the gender in the mandibular left 1st molar and both molars on the right side with p value of <0.001.

Pulp volume did not show any significant changes among the different age groups. Linear regression analysis shows that the equation Age= 21.243 + 1.127 x ln (pulp volume36) +10.654 x ln (pulp volume 37)-10.721x ln(pulp volume 46) +0.163 x ln(pulp volume 47) has an R value of 0.427 and SEE of 6.27 years for prediction of age which was statistically insignificant.

Table I: Independent T Test for comparison of the TCI in male and female

<table>
<thead>
<tr>
<th></th>
<th>FEMALE(n=56)</th>
<th>MALE(n=44)</th>
<th>t</th>
<th>df</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Sd</td>
<td>Mean</td>
<td>sd</td>
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<td>34.191</td>
<td>9.049</td>
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<td>34.775</td>
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<tr>
<td>TCI 47</td>
<td>25.479</td>
<td>8.818</td>
<td>26.829</td>
<td>8.614</td>
<td>-0.768</td>
</tr>
</tbody>
</table>
Fig I: Cone beam computed tomography images of the mandibular left molar obtained in the sagittal section shows the measurement of CH (red line) and CPCH (yellow line).

Fig II: Cone beam computed tomography images showing the pulp volume of mandibular left molar obtained in 3D rendered view (axial, coronal, and sagittal views)

Table I: Independent T Test for comparison of the TCI in male and female

<table>
<thead>
<tr>
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<td>8.614</td>
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</tr>
</tbody>
</table>

Fig I: Cone beam computed tomography images of the mandibular left molar obtained in the sagittal section shows the measurement of CH (red line) and CPCH (yellow line).
Discussion

Tooth coronal index aids in age estimation in people whose age is unknown for a variety of reasons like, minor children are made to work for various reasons, among people who migrate for various reasons, in natural disasters and also from archaeological specimens.

Secondary dentine is formed due to wasting diseases (erosion, abrasions and attrition), ageing, caries etc. which gradually results in the decrease in the pulp volume. Secondary dentine is considered as an age predictor. As age advances the pulpal volume decreases due to apposition of secondary dentine along the dentinal walls. The second reason being that it can withstand damages caused due environmental factors in human remains as it is encased in a harder tissue. This deposition of secondary dentine is not uniform and can vary in different parts in the pulp chamber.

Pulp cavity size decrease as age advances due to secondary dentine getting deposited along the roof and floor thereby reducing the height rather than the width. Pulpal changes were also seen after orthodontic treatment. This decrease in pulpal height acts as a biomarker in age estimation. Sectioning of tooth and radiographs can be used for measuring secondary dentine deposition.

CBCT, a three dimensional imaging modality has advantages over 2 D imaging as it provides images of tooth without magnification, distortion, no superimpositions and images can be viewed in multiple sections at multiple levels. Due to the spatial resolution of CBCT, it can be used to visualise the pulp chamber and anatomic variations. Radiation dose is relatively less when compared to other 3 D imaging modalities.

In our study we measured both coronal and pulp height in sagittal section as it was the ideal section for measuring the maximal height of both tooth and pulp chamber when compared to other sections.

Comparing the TCI value between the genders showed no significant results which was similar to the study done by Drusini AG; Khattab et al.2017 and Nawaya and Burhan 2016; but was not in accordance with the studies done by Igbigbi P and Nyirenda SK 2005, they found that gender had significance influence on age using TCI. Few studies support this theory explaining that it could be due to the influence of estrogen in secondary dentine deposition.

In our study using images taken in CBCT we found that the mandibular right molars (46,47) the TCI values corelated well with age and it was found that the lower right first molar (46) showed a significant prediction of age in females. This was the best predictor in the equation, which was in accordance to a study by Agematsu H & El Morsi DA 2015. As R value is positive it shows a positive correlation indicating that...
as age increases the index increases and also it was more in females when compared to the males which was similar to the study done by EL Morsi DA 2015 which could be related to the hormonal influence, which could lead to increase in the sequential obliteration of the pulp with growth.

Pulp volume measurement was not statistically significant; it could be because the measurements were taken from images of patients with an age range of 15-30 years. But in our study there was a significant change in the volume of pulp when compared among both sexes. The volume of the pulp chamber was stronger for the males in the present study which was in accordance to a study done by Agematsu H et al. 14.

**Conclusion**

CBCT plays an important in age as it gives a more accurate picture regarding the dimensions in all planes without with less radiation dose. CBCT could prove useful in forensic odontology as important diagnostic tool in age and sex determination in living and also aid in diagnosing the age and gender of victims in mass disasters. In present study TCI was useful in in age estimation this method was not useful in gender determination.

**Ethical Clearance**- Taken from, Institutional Ethics Committee Manipal College of Dental Sciences, Mangaluru IEC Protocol Ref No.18070

**Source of Funding**- Nil

**Conflict of Interest**- Nil

**References**

Original Research Article

A Cross-sectional Radiological Study of Ossification at Lower End of Ulna and Tip of Olecranon among Children and Adolescents coming to Forensic Medicine Department, Medical College, Kolkata, for Age Estimation

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Abstract

Objectives: To study radiologically the ossification status of lower end of ulna and tip of olecranon and compare with Galstaun chart and other standard charts.

Material and Methods: A record based cross sectional study was performed in the department of Forensic and State Medicine, Medical College Kolkata for one month, on children and adolescent coming to the department for age estimation over a span of past one year. Their chronological age was noted from birth certificates. The X-Ray plates was looked for appearance and fusion of lower end of ulna and tip of olecranon and compared with Galstaun chart and other standard charts.

Results: The records of 199 subjects (Male-142, Female-57) were examined, who belong to the age group of 7 yrs to 19 yrs. After observing the appearance and fusion from X-Ray plates and comparing them with Galstaun chart it was seen that maximum deviation from mean age occurred in appearance of lower end of ulna. Appearance of tip of olecranon also had a wide deviation. Males were found to have more variations than females.

Keywords: Age estimation, Radiological study, ossification, children, adolescent.

Introduction

Age estimation is one of the regular assignments in clinical forensic medicine. As illiteracy is a major drawback in India, many citizens are unaware of the importance of registration of birth and death. Even if there is registration, there is often lack of maintenance of records. That’s why forensic age estimation becomes important in certain civil and criminal issues. Though there are many methods of age estimation, namely physical (general development), dental (eruption of teeth and root calcification sometimes aided by Gustafson’s method) etc., radiological estimation by observation of time related appearance and fusion of different secondary ossification centers, is the commonest method used. It is reliable upto 22-25 years of age only, as mentioned by most of the authors.¹ Age, for the appearance of ossification centers, is relatively constant with minor variations. There are many factors which influence the appearance and fusion of bony centers like climatic, economic, hereditary, dietetic, geographical and some other unknown factors.¹

The Indian population differs from the western population in different anthropological traits. Studies done on this subject in India are inadequate. Galstaun in 1930 and 1937 has done a study on Bengali population. The Galstaun chart is the most widely used chart of its kind for comparison, in most parts of West Bengal. According to this chart the age of appearance of ossification centre for lower end of ulna in male is 10 to 11 yrs and in females is 8 to 10 yrs; and the age of fusion for the same in males is 18 yrs and in females is 17 yrs respectively. Similarly the age of appearance of ossification centre for tip of olecranon in males is 11 to 13 yrs and for females is 9 to 12 yrs and age of fusion for males is 17 yrs and for females is 15 yrs respectively.³
Likewise Bajaj studied on Delhi population in 1967, Pilai in Madras in 1936, Basu & Basu on Bengalis in 1938, Agarwal & Pathak on Punjabis in 1957 etc. [2] Most of the data for those studies were collected using traditional X-Ray. The present study focuses on observation of status of ossification of secondary ossification centers using digital x-ray and applying traditional comparison method. Determination of the age of an individual from the observation of appearance and fusion of the secondary ossification centers is legally and medico-legally accepted in India. The present study was carried out by observing the epiphyseal appearance and union at the lower end of ulna and tip of olecranon. This study was carried out in subjects (children and adolescents) who attended forensic medicine department of Medical College, Kolkata for estimation of age in civil matters (recruitment in age-category sports).

**Aims and Objectives**

1. To study the radiological appearance and fusion of secondary ossification centers in lower end of ulna and tip of olecranon

2. To assess whether there is any significant difference between the radiological bone age of today’s population with that of standard charts (Galstaun)

3. To find out the gender wise disparity if any

4. To compare the results with other studies done in India

**Material and Methods**

Study type (Design) – Record based cross-sectional study (Descriptive)

Study period – 1 month (15th December 2019 to 15th January 2020)

Study population- All the children and adolescent from native Bengali population who came to the Upgraded Department of Forensic and State Medicine of Medical College, Kolkata for determination of age.

Place of study – Upgraded Department of Forensic and State Medicine, Medical College, Kolkata

Sample size – 199; Male-142, Female-57

**Inclusion criteria-**

1. All the children and adolescent from native Bengali population residing at sub-urban areas of Kolkata, who came to the forensic and state medicine department of Medical College Kolkata for age determination for the purpose of recruitment in age category sports

2. Sex- all inclusive

3. Age – all inclusive

4. Must have age proof in the form of birth certificate and mothers hospital discharge certificate

5. Must have residential proof in the form of residential certificate

**Exclusion criteria –**

1. Individuals having skeletal or craniofacial deformity.

2. Individuals suffering from hormonal disorders affecting growth and development.

3. Individuals suffering from malnutrition and other nutritional disorders affecting growth and development

**Study tools :**

1. Digital skiagram showing antero-posterior view of wrist joint and antero-posterior and lateral view of elbow joint of non-dominant hand.

2. View box

3. Standard charts (of radiological appearance and ossification of secondary ossification centers)

4. Technical features of digital X-ray machine-generator of 80 KW (kilowatt) power, collimator with focal spot size of 0.6mm, anode heat capacity of 300 KHU (Kilo Heat Unit where 1 Joule =1.4 HU), ceiling suspended tube with movements in all direction, x-ray table having a weight bearing capacity of 200 kg, digital detector with a spatial resolution of 2.5 lines pair/millimeter, high speed processor of 32 bit and an image storage disc of 70 gigabyte.

**Study technique :**

All cases recorded during the last one year from the study period were considered for study after observing the inclusion and exclusion criteria. Digital
x-ray of wrist and elbow joint of non dominant side were taken for observation. Records of preliminary physical examination were looked for to exclude any apparent growth retardation, malnutrition and other exclusion criteria. The x-ray plates were examined for the appearance and fusion of secondary centers of ossification in respect to lower end of ulna and tip of olecranon and compared with the Galstaun chart for known chronological age. The age was estimated using the conventional comparison method. As a convention and regular practice in the department of Forensic and State Medicine, Medical College Kolkata each and every x-ray plate was interpreted by a team of four members comprising of one senior faculty, one junior faculty and two residents. This method was applied to minimize interobserver variation as collective decision (consensus) of the team was taken into account for interpretation. So the data in the record was actually an interpretation by a team of forensic experts. Moreover, each and every plate was re-examined by the authors. However no statistical analysis was done to measure the interobserver variation and this is mentioned in the limitation of study. For appearance, “just appeared” was also taken into account and for fusion, “complete fusion” was taken into consideration. The results were then statistically evaluated after tabulating the collected data in excel-sheet.

Consent for the study:

As it is a record based study on subjects coming for age estimation in relation to qualification in age category sports, no separate consent was taken, as consent was given during medical examination for using the data for academic purposes in the future.

Review of Literature

There appears to be limited number of studies in India related to age estimation using routine x-rays and quite fewer in West Bengal. Age estimation, being an important aid in legal matters, demands accuracy and so it becomes pertinent to follow the latest data related to a particular population. But unfortunately in most of the set ups age old data chart is still in use.

In one of the studies which was carried out on 104 subjects, 54 males and 50 females, in the age group of 15-21 yrs, in B.J Medical College, Ahmedabad in 2009, using x-rays of lower end of radius and ulna, it was seen that completion of epiphyseal fusion in lower end of ulna of both hands in males is 19 to 20 yrs and for females is 18 to 19 yrs.

Another study done in the department of forensic medicine of Surat Medical College, using x-rays of lower end of radius and ulna, among 218 cases of age group of 14 to 21 yrs, was of the conclusion that fusion of lower end of ulna started at 14 to 15 yrs and completed at 19 yrs in female and 21 yrs in male.

In a study on epiphyseal union at elbow and wrist joints in the radiology department of King George Hospital, Lucknow among Indian females of age group 10 to 18 yrs, showed that the results were about at least 2 years ahead of the figures given in most of the textbooks.

Department of Anatomy of Government Medical College, Nagpur carried out a study on ages of epiphyseal union around wrist joint among 80 subjects (44 girls and 36 boys) of age group of 13 to 23 years; lower end of ulna was found to be completely fused at 19 to 20 years in both males and females.

A radiological study of epiphyseal union of distal end of radius and ulna in subjects of age group 16 to 22 yrs was carried out on 100 healthy medical students (50 males &50 females) of Dr. S.N. Medical College, Jodhpur (Rajasthan). In the same study average age for complete epiphyseal fusion of lower end of ulna for males is 19 to 20 years and for females is 18 to 19 yrs.

In a prospective cross sectional hospital based study, conducted in Sudan, with data collected from 5 hospitals; Khartoum, Khartoum North, Omdurman, Police, and Um badda, 57 (Fifty seven) AP and Lateral plane elbow x- rays were taken for healthy Sudanese children (40 Males and 17 Females), aged 13 to 23 years. In this study, the films of the 1st group (at average of 14 years) only 20% of them showed closed olecanon process. In the 2nd group (at average of 17 years) 66.7% showed closed olecranon process. All children above 19 yrs (3rd group) had closed physeal. The female physeal fusion precedes the male in one of all the 6 centers.

The discrepancies in so many studies have heightened the need for a digital skiagram based study
to focus on the need of a region based data for age estimation using x-ray plates.

**Results**

Out of 250 recorded subjects, 199 were included keeping in mind the inclusion criteria, in which 142 were males and 57 females. There are variations in appearance of lower end of Ulna in both males (Table I) (Fig. 2) and females (Table II) where there are frequencies of appearances below the given age range by standard charts. Similarly the fusion for lower end of Ulna occurred before the stipulated age range of standard charts in males (Table III). But in all females the fusion of lower end of ulna occurred by 17 yrs.

Frequency of tip of olecranon appearance in males (Table IV) and females (Table V) (Fig.1) also showed variations from Galstaun chart. Similarly the secondary centers for tip of olecranon showed earlier fusion than the standard age according to Galstaun in both males (Table VI) and females (Table VII). The comparison of radiological bone age of this study with that of Galstaun chart (Table VIII) for both the secondary centers of ossification clearly depicts the variations. The percentage for conformation in appearance of the ossification centers in both males and females (Table IX) with Galstaun chart showed that the female age ranges have less variations than males, however, the percentage for conformation in fusion with the Galstaun chart showed a mixed variation. A comparison with other charts followed in India (Table X) heightens the need for updating the standard charts.

**Table no I: Frequency of lower end of Ulna appearance in males (n = 142)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Status</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 y</td>
<td>Appeared</td>
<td>37</td>
<td>26.1</td>
</tr>
<tr>
<td>10-11 y</td>
<td>Appeared</td>
<td>105</td>
<td>73.9</td>
</tr>
<tr>
<td>&gt;11 y</td>
<td>Not appeared</td>
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</table>

**Table no II: Frequency of lower end of Ulna appearance in females (n = 57)**

<table>
<thead>
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<th>Age</th>
<th>Status</th>
<th>Numbers</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8 y</td>
<td>Appeared</td>
<td>2</td>
<td>3.5</td>
</tr>
<tr>
<td>8-10 y</td>
<td>Appeared</td>
<td>55</td>
<td>96.5</td>
</tr>
<tr>
<td>&gt;10 y</td>
<td>Not appeared</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table no III: Frequency of tip of Olecranon appearance in males (n=142)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Status</th>
<th>Numbers</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11 y</td>
<td>Appeared</td>
<td>28</td>
<td>19.7</td>
</tr>
<tr>
<td>11-13 y</td>
<td>Appeared</td>
<td>114</td>
<td>80.3</td>
</tr>
<tr>
<td>&gt;13 y</td>
<td>Not appeared</td>
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<td>0</td>
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</tbody>
</table>
Table IV: Frequency of tip of olecranon appearance in females (n=57)

<table>
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<th>Age</th>
<th>Status</th>
<th>Number</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>&lt;9 y</td>
<td>Appeared</td>
<td>4</td>
<td>7.0</td>
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<tr>
<td>9-12 y</td>
<td>Appeared</td>
<td>53</td>
<td>93</td>
</tr>
<tr>
<td>&gt;12 y</td>
<td>Not appeared</td>
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</table>

Table V: Radiological bone age in our study compared to standard Galstaun chart

<table>
<thead>
<tr>
<th>Secondary centers of ossification</th>
<th>Age range of our study (y – years)</th>
<th>Galstaun’s age range (y-years)</th>
<th>Gender</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower end of ulna</td>
<td>8-11 y</td>
<td>10-11 y</td>
<td>M</td>
<td>Appearance</td>
</tr>
<tr>
<td>Lower end of ulna</td>
<td>7-10 y</td>
<td>8-10 y</td>
<td>F</td>
<td>Appearance</td>
</tr>
<tr>
<td>Tip of olecranon</td>
<td>9-13 y</td>
<td>11-13 y</td>
<td>M</td>
<td>Appearance</td>
</tr>
<tr>
<td>Tip of olecranon</td>
<td>7-12 y</td>
<td>9-12 y</td>
<td>F</td>
<td>Appearance</td>
</tr>
<tr>
<td>Lower end of ulna</td>
<td>17-18 y</td>
<td>18 y</td>
<td>M</td>
<td>Fusion</td>
</tr>
<tr>
<td>Lower end of ulna</td>
<td>17 y</td>
<td>17 y</td>
<td>F</td>
<td>Fusion</td>
</tr>
<tr>
<td>Tip of olecranon</td>
<td>15-17 y</td>
<td>17 y</td>
<td>M</td>
<td>Fusion</td>
</tr>
<tr>
<td>Tip of olecranon</td>
<td>14-15 y</td>
<td>15 y</td>
<td>F</td>
<td>Fusion</td>
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Table VI: Comparison with other standard charts of India

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<th>Studies</th>
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<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>F</td>
</tr>
<tr>
<td>Hepworth</td>
<td>-</td>
<td>16-17</td>
</tr>
<tr>
<td>Lall R &amp; Nat B S</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>Pillai</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>Basu&amp;Basu</td>
<td>-</td>
<td>16-17</td>
</tr>
<tr>
<td>Mittal</td>
<td>-</td>
<td>17-18</td>
</tr>
<tr>
<td>Our study</td>
<td>8-11 y</td>
<td>17-18 y</td>
</tr>
</tbody>
</table>
Discussion

There are limited number of studies in India related to age estimation using routine x-rays and quite fewer in West Bengal. As Age estimation is an important aid in legal matters, it becomes pertinent to follow the latest data related to a particular population. But unfortunately in most of the set ups age old data chart is still in use.

In the present study, total number of subjects taken into consideration from the records were 199 (n= 199), out of which 142 were males and 57 were females. The results for appearance of lower end of ulna in males showed that for 26.1% of male subjects the appearance occurred before 10 years whereas in Galstaun chart, which is followed in West Bengal the age for appearance of secondary ossification centre of lower end of ulna is 10 to 11 years[3]. But in females, 3.5% of the subjects showed appearance before 8 years, the age of appearance of lower end of Ulna according to Galstaun being 8 to 10 years. The fusion of lower end of Ulna in males in 99.3% of cases occurred at 18 years which is in accordance with the Galstaun’s age range and in females all showed fusion by 17 years of age which is at par with the Galstaun chart.

The age of appearance of tip of olecranon in 19.7% of males occurred below 11 years of age and in the rest it appeared at 11 to 13 years of age which is the age range mentioned in Galstaun chart and in 7.0% of females the appearance occurred below 9 years, the age according to the galstaun chart being 9-12 years. In 5.6% of males, the tip of olecranon was found to have fused below 17 years and in the rest 94.4% it fused at 17 years in accordance with Galstaun chart and in 12.3% of females the fusion occurred below 15 years and in the rest 87.7 % the fusion was at par with the Galstaun chart that is at 15 years of age.

Hepworth et al. in his study found the age for lower end of ulna fusion in males and females to be 16-17 years which is similar with the results of study by Basu & Basu, which is almost consistent with the results of our study. However Pillai stated that the age for fusion of lower end of ulna in both males and females is 18 years.[2]

In results of the studies which were carried out in BJ Medical College, Ahmedabad (2009), Department of forensic medicine of Surat Medical College, Department of Anatomy of Government Medical College, Nagpur, Radiology department of King George Hospital, Lucknow and Dr. S.N. Medical College, Jodhpur (Rajasthan), are inconsistent with our study results. The discrepancies in so many studies demands the need for a digital skiagram based study to focus on the need of a region based data for age estimation using X-Ray plates.

Conclusion

The appearance of lower end of ulna and tip of olecranon in both males and females vary significantly from GALSTAUN CHART as well as from other studies. The fusion of the lower end of ulna and tip of olecranon in both males and females is almost in accordance with other studies. The variation can be due to the factors affecting the ossification like geographical, environmental, hereditary factors etc. The uniqueness of the finding of the study lies in the fact that it is one of the very few studies to include tip of olecranon on native Bengali population and in our study fusion was less variable than the appearance, which is quite contrary to the general rule.

Limitations:

1. The other factors affecting bone age were not taken into consideration like geographical, genetic, nutritional factors etc.

2. Larger sample size is preferable in further studies

3. A certain amount of children from a locality might not represent the whole population of said age group of that community.

4. There might be considerable interobserver variation in the interpretation of x-rays in such type of observational studies. Every effort was made to minimize interobserver variation while designing the study, through the process of collective observation (i.e. consensus) by a group of forensic experts and re-examination by the authors. However, the interobserver variation was not measurable. Still some amount of interobserver variations cannot be ruled out.

5. Further study regarding different stages of fusion is preferable
Recommendations:

- To formulate separate standards of ossification for geographically distinct regions
- To carry out further studies on a larger sample size
- To update the age old standard charts

Conflict of Interest: Nil

Source of Funding: The study was conducted within the set-up of a state government medical college with the help of self funding when and where required.

Ethical Clearance: Ethical clearance and approval taken from the Institutional Ethics Committee of Medical College, Kolkata.

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Determinant of Incompliance Medication People with Tuberculosis Disease

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Abstract
Indonesian Tuberculosis (Tb) case is the highest third rank in the world. It is caused by disobedience in therapy. Three factors affect medication compliance include predisposing, reinforcing and enabling factors. This research aims to analyzed factors that affects medication compliance in Indonesian Tb patients. The study was analytical research. The population were 119 Tb patients in Surabaya Health care center, Indonesia. Simple random sampling technique was used to take sample, consist of 93 respondents. The variables of this study were predisposing factors, reinforcing factors and enabling factors. The data was collected with questionnaires and analyzed with chi square test. The study showed that all factors related to incompliance medication people with Tb, namely predisposing factors: knowledge, attitude, belief (p=0.000), trust (p=0.013, and values (p=0.001); reinforcing factors: family support (p=0.034) and healthcare personnel support (p=0.022); and enabling factors: healthcare facility (p=0.000) and physical environment (p=.000). The determinants of incompliance medication people with Tb include predisposing factors (knowledge, attitudes, beliefs, trust and values); reinforcing factors (family and healthcare personnel support); and enabling factors (healthcare facility and environment). Tackling incompliance medication on Tb should involve private sector, family sector, society/healthcare sector and government sector.

Keywords: Tuberculosis, predisposing, reinforcing, enabling

Introduction
The WHO reports there are three issues related to tuberculosis. The first issue is high pain and mortality figures due to Tb. Tb became the second largest disease killer infection. The second issue is that almost three million Tb cases are not detected by the health system, both undiagnosed and diagnosed but not checked. The third issue is the increase of MDR-TB case[1]. Indonesia has a free TB program in 2035, with efforts among others: ensuring that 95% of TB cases get treatment and increased 90% of treatment success[2].

The Millennium Development Goal targets for 2015 of reducing TB prevalence and deaths by half globally has made some impact on the burden of targeted diseases, including TB. The number of TB cases in Indonesia is in the ranks of three worlds after India and China[3]. On the national scale, the number of TB cases in East Java was at the rank of two after West Java. Based on the results of a survey in Healthcare Surabaya in September 2018 was obtained the number of TB sufferers of 121 people, this number is included from the number of patients with lung tuberculosis both children and adults. Based on the observation data obtained by 10% sufferers are reminded by PMO (supervision of drug swallowing), then by 15% sufferers experience a drug drop out, and as high as 3% died due to TB.

Factors affecting one’s adherents as discussed in Precede and proceed theories by Lawrence Green include predisposition factors, supporting factors and reinforcing factors[4]. The predisposition factor is a factor that exists in a person, among them the knowledge, attitudes, beliefs, and values that a person believes strongly affects
how the person’s behavior is to obey in taking medicine. Determining the success of tuberculosis therapy is one of the patient’s compliance with therapy. Compliance is a behavior that complies with the order to comply with the regulations. In achieving the objective of compliance with TB drugs need to be used to become a living norm and culture of TB sufferers so conscious and self-reliant for healthy living. However, growing an awareness of compliance with TB drug, need an action that can motivate correctly and consistently.

Therefore, it is important for people with TB to complete the therapeutic program well. In other words, adherence to the sufferer for the cure of TB disease is useful to improve patient’s compliance in taking medicine, it is necessary information addressed to the sufferer on how important it is to take medication, give confidence in healing, and provide consultation for the sufferer and family.

**Materials and Methods**

This Design research is an analytical study using the cross-sectional approach. The population in this study is the patient with lung tuberculosis who came to medicine at the Perak East Surabaya Health Center for 119 people. A large sample is 93 respondents were taken using probability sampling techniques with simple random sampling. Independent variables in this study are predisposing factors (knowledge, attitudes, beliefs and values); Enabling factor (health facilities, environment); Factor reinforcing (living support and healthcare personnel support) and dependent variables are adherence to taking medication. Data collection instruments using questionnaires with a check list. Data analyzed using Chi square test.

**Results and Discussion**

**Table 1. Characteristics of participants’ socio-demography (n=93).**

<table>
<thead>
<tr>
<th>Socio-demography</th>
<th>Category</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Children</td>
<td>9</td>
<td>9,8%</td>
</tr>
<tr>
<td></td>
<td>Teen</td>
<td>15</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>40</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>Elderly</td>
<td>29</td>
<td>31,2%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>54</td>
<td>58%</td>
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<tr>
<td></td>
<td>Female</td>
<td>39</td>
<td>42%</td>
</tr>
<tr>
<td>Education level</td>
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<td>18%</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>47</td>
<td>51%</td>
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<tr>
<td></td>
<td>Top</td>
<td>24</td>
<td>26%</td>
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<td>5%</td>
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<td>55%</td>
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<td></td>
<td>Work</td>
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<td>suffering from TB</td>
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<td>Category 3</td>
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<td>24%</td>
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</table>
Table 2. Determinant of Incompliance Medication People with Tuberculosis Disease (n=93).

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<tr>
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<td>F</td>
<td>%</td>
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<td>17</td>
<td>39.5</td>
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<td>1</td>
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<tr>
<td>Good</td>
<td>60</td>
<td>87</td>
<td>9</td>
<td>13</td>
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</tr>
<tr>
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<td>15</td>
<td>68.2</td>
<td>7</td>
<td>31.8</td>
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<tr>
<td>Poor</td>
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<td>50</td>
<td>1</td>
<td>50</td>
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</tr>
<tr>
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<td>22</td>
<td>73.3</td>
<td>8</td>
<td>26.7</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enough</td>
<td>40</td>
<td>83.3</td>
<td>8</td>
<td>16.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>14</td>
<td>93.3</td>
<td>1</td>
<td>6.7</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Good</td>
<td>32</td>
<td>84.2</td>
<td>6</td>
<td>16.8</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enough</td>
<td>31</td>
<td>79.5</td>
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Factor predisposing

Predisposing factors are defined as personal characteristics or populations that motivate individuals in adopting certain health behaviors; This includes knowledge, values, and attitudes that may explain individual behavior[4].

Knowledge is one of the domains of behavior formation in the treatment of tuberculosis[5]. Patient knowledge needs to be improved, especially knowledge about treatment procedures (how to take medication, drug side effects and taking the drug until complete is not interrupted)[5,6,7].

Patients’ lack of comprehensive information about Tb and its treatment at the start of treatment make incomplete treatment[8]. The more information received can increase the knowledge of TB sufferers and the more obedient to take medication. Compliance is a person’s obedience to a commandment, self-awareness is increasingly open with only the information obtained. The increase in knowledge is not absolutely obtained in formal education, but also can be obtained in non-formal education such as, counseling, health seminars, and health counseling[9].

Attitude has not been an action (open reaction) or activity, but it is a predisposition of behavior (action), or a closed reaction is not a readiness to react to objects in a particular environment as an action to the object. Attitudes have a significant influence on the obedience of a person in taking medicine. Attitudes are an underlying form to be willing and submissive in this respect in taking the medicine. Exposure that is often given to a person affects the individual in decision-making and acting. The compliance attitude of taking medication and having a diet has the opportunity to heal very large. While disobedience can extend the pain and increase the severity of the disease[9].

Belief is one’s willingness to rely on others where he has faith in him. Trust is a mental condition based on a person’s situation in the social context. Healthy lifestyle is the choice that one has for his life for those who believe that the disease comes from a wrong lifestyle will always keep his lifestyle to be healthy. In line with the health theory belief models that trust can affect health.

Tb was curable. This belief needs to be matched to the patient, so that the patient has the motivation to heal by undergoing complete treatment[10].

Values are freely selected, containing beliefs or behaviors about the meaning of a person, object, idea, or action. Value is crucial because it affects decisions and actions. The linked value of compliance in taking the drug will increase the value of the device to a priority. Berman (2014) mentioned that the device value is a small group of values embraced by individuals. Individuals regulate their value devices along the continuum, ranging from the most important to the least important, forming a value system. The value system is the foundation of the path of life, gives direction in life, and forms the basis of behavior, especially behaviors based on decisions or choices[11]. TB sufferers who are undergoing treatment have moderate stress due to taking the drug for a long period[12].

Factor reinforcing

Family support is one of the factors affecting medication compliance. Family support includes instrumental, informational, appraisal, emotional support. The family plays a role as a supervisor to take medication and reduce stigma in family and society[5,13]. Family support includes the patient’s motivation to undergo treatment, to be a medical supervisor and provide support in accessing health care to obtain anti-TB medications. Caring for TB sufferers is a stressor for TB caregiver at home. Caregiver TB not only acts as a drug-taking supervisor. Caregiver TB should be able to manage stress while caring for TB sufferers to avoid contracting easily[14]. Stress condition if not immediately resolved will increase cortisol levels to suppress immune system caregiver TB and easy to get displaced[14,15].

The support of healthcare officers (physicians and nurses) is an important factor in treatment of tuberculosis. Activities undertaken by the health Officer include Health promotion (counseling related to prevention of infection), curative (treatment process, side effects of the drug, and taking medication until complete)[7,16]. The challenge for health workers is how patients and families will undergo complete treatment, including attitudes (friendliness, empathy, and attention).
Factor enabling

Enabling factors, such as access to services or support, which can facilitate the adoption of a particular behavior\[5\], type of facility, type of providers at first visit, number of visits, number of providers consulted before reaching a TB diagnosis and expenses plus travel time were assessed\[17\]. Health facilities that are available in Puskesmas Surabaya is very adequate, among others, the availability of drug flow, health promotion brochure, comfortable waiting room, and a clean toilet. It is also a factor that affects patients to come remediation\[18\]. As a form of support to the TB program, the government of Indonesia has secured all TB patient financing through the Social Security Administering Agency (BPJS) since 2014\[19,20\].

Conclusion

In conclusion, the determinants of incompliance medication people with Tb include predisposing factors (knowledge, attitudes, beliefs, trust and values); reinforcing factors (family and healthcare personnel support); and enabling factors (healthcare facility and environment). Tackling incompliance medication on Tb should involve private sector, family sector, society/healthcare sector and government sector.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: This study supported by the Ministry of Education and Culture of the Republic of Indonesia and Rector Universitas Nahdlatul Ulama Surabaya.

Acknowledgements : We thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study had been approved by the Faculty of Nursing and Midwifery, Universitas Nahdlatul Ulama Surabaya.

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REVIEW ARTICLE
COVID-19 – Current Status and Trends: A Comprehensive Review

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Abstract
The current global pandemic of COVID-19 is caused by a virus of corona family named SARS-CoV-2. Cardiovascular manifestations of COVID-19 are varied and complex and include myocarditis, acute coronary syndrome, heart failure, pericarditis and pericardial effusion. Those with cardiac comorbidities are at higher risk of severe infection and death. SARS-CoV-2 may infiltrate and injury the heart directly or may cause Myocardial infarction, LV dysfunction secondary to myocarditis, congestive cardiac failure, rhythm abnormalities and pulmonary and other vascular embolisms. The medications used to treat COVID-19 may also have serious cardiac side effects. A through understanding of the cardiac involvement of COVID-19 and its varied presentation is needed for management of COVID-19 infection and its complications.

Keywords COVID-19, myocarditis, myocardial injury

Introduction
Coronavirus disease 2019 (COVID-19) is a disease syndrome caused by the novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a single stranded positive sense RNA virus belonging to the family Coronaviridae[1, 2]. The current pandemic of COVID-19 is one of the greatest threats faced by humanity in the 21st century. This disease, identified by Dr Zhang Jixian from Hubei Provincial Hospital had started initially in Wuhan, China in December 2019[1] but has then spread rapidly and on March 11 was declared a pandemic. As of October 1st, 2020 the virus has spread to countries with 33 million cases and one million deaths of COVID-19 have now been reported globally.[3] COVID-19 is extremely contagious and if precautions not taken can infect 60-80% of the population.[4]

COVID-19 can have variable presentation ranging from asymptomatic infection, Mild sore throat to severe pneumonia with Acute Respiratory Distress Syndrome, Multi Organ Dysfunction to death.[5] The lungs have been the main involvement seen in COVID-19 infection, however in 27-40% cases, cardiac involvement is also seen and has a worse prognosis in them.[6-8] Earlier studies have shown cardiac involvement in previous influenza and coronavirus epidemics, including myocarditis, acute myocardial infarction, and worsening of heart failure leading to significant morbidity and mortality.[9] COVID related myocarditis has been described with varied electrocardiographic changes, rise in troponin and echocardiographic evidence of systolic dysfunction. Patients with pre-existing comorbidities are thought to be at an increased risk of infection with SARS-CoV2 and also tend to have worse clinical outcomes[8]. Myocardial injury is associated with cardiac dysfunction and arrhythmias and death compared to those without cardiac injury.[10]

In this article we have reviewed available literature to describe cardiac involvement in COVID-19 with respect to the risk factors, comorbidities, pathophysiology
and complications associated with the infection. This review aims for physicians and cardiologists to update the knowledge of the pandemic and assist them in management of the same.

CARDIOVASCULAR INVOLVEMENT IN COVID-19

Epidemiology

Age

The severity of COVID infection and complications increase with age.[11]. In the elderly age group of 75 to 84 years the rate of admission to ICU was 11 to 31% while in less than 20 years of age the risk of hospitalization was 2 to 3%[12]. Increasing age was also associated with increased mortality with death rate up to 27.3% in the elderly age group and 0.1 to 0.2% in the younger age group[13]. There have also been reports of increased incidence of Kawasaki disease noticed in children with COVID-19 infection.[14]

Sex

Higher Hospitalization rates, more complications and mortality was noted in males compared to females[11]. Studies have shown male preponderance ranging from 52.9% to 60% of the hospitalized patients[11, 15]. The mortality rate in men was higher than women for every age group. The pathophysiology and significance of male predominance of COVID-19 disease is uncertain.

Comorbidities

Patients with pre-existing co-morbidities are at an increased risk of infection with SARS-CoV2 and tend to have worse clinical outcomes[16]. In a study by Wang et al it was reported that in patients with severe disease 25% had cardiovascular diseases, 44% had arrhythmia, and 58% had hypertension.[17] Notably a high complication rate with mortality rate of 10.5% was reported in cardiac patients while hypertensive patients had 6.0% death rates and diabetic patients had 7.3% death rates.[6] In a review of 1590 patients by Zhou et al, those with comorbidities of hypertension, diabetes, heart disease had severe COVID-19 disease requiring ICU care and increased mortality.[18] Recent studies have suggested that obesity may also be a risk factor for severe COVID-19 disease.[15, 19]

Virology and entry into human host cell

Coronaviridae (CoV) are so called because of their crown like appearance. The COVID-19 pathogen belongs to the β-CoV group. CoV has four major structural proteins: the nucleocapsid (N) protein, envelope (E) protein, the membrane (M) protein, and the spike (S) protein which allows attachment and fusion with the host receptor.[20] The animal reservoir of COVID-19 is presently uncertain, but the viral genome was found to be approximately 88% similar to Bat coronavirus and distant to the previously known SARS and Middle East respiratory syndrome viruses.[2] The entry of virus into the human cell followed by the pathophysiology of COVID infection is demonstrated in figure 1.

Mechanism of cardiovascular Involvement

SARS-CoV-2 can bind to the angiotensin-converting enzyme 2 (ACE2) receptor and enter the host cell.[21] These receptors are highly expressed in the heart and lungs, and they have been confirmed to be the functional receptors for the novel coronavirus.[22] Hence The virus infiltrates the human cells through angiotensin-converting enzyme 2 (ACE2) receptors which leads to ARDS, myocarditis and cardiac failure.[23] This receptor may be upregulated in people taking ACE inhibitors and ARBs, thus theoretically providing more targets for the virus’ spike protein, which binds to the ACE-2 receptor. Due to this pathway there has been a lot of discussion on use of ACE inhibitors for hypertension whether they make patients susceptible to SARS-CoV-2 infection. However, there is no clear evidence that ACEIs or ARBs may increase the odds of virus entry into host cells. Hence updates from the American heart association, European Society of Cardiology and European Society of Hypertension suggest that ACE inhibitors should be maintained or initiated as per need in patients with myocardial infections, heart failure, or hypertension.[24]

COVID-19 and myocarditis

Acute myocarditis is a known complication of any acute viral infection. Cardiac muscle autopsy specimens have shown signs of myocarditis such as mononuclear cell infiltrates and myocardial necrosis.[25, 26] Acute myocardial injury is the most common cardiovascular complication inCOVID-19 manifested by elevation of high-sensitivity cardiac troponin I and the incidence of
acute myocardial injury has been reported to be around 8% to 12%. These studies suggest that fulminant myocarditis is an important cause of the acute cardiac injury in COVID-19 patients. The symptoms may vary from angina, breathlessness on exertion, tiredness to cardiac failure, shock, rhythm disturbance and sudden cardiac death. Varied arrhythmias ranging from AV blocks to atrial and ventricular tachyarrhythmias. Transient ECG changes are common and may even mimic a ST Elevation MI but coronary angiogram is usually not showing any obstructive lesion. Up to two to three times elevation of cardiac troponin levels is suggestive of previous cardiac conditions or mild cardiac injury cardiac condition and/or acute injury related to COVID-19 whereas more than five times elevations is suggestive of myocarditis, myocardial infarction secondary to plaque rupture and systemic thrombosis. Serum high-sensitivity troponin, High BNP/NT-proBNP and Hs CRP levels correlate with the extent of ventricular stress and also the severity of illness. Elevations of DDimer have been associated with poor outcome. The rise in Tn-I does not occur in isolation but alongside the rise of other inflammatory markers, such as ferritin, c-reactive protein, interleukin-6 [IL-6], interferon-γ, tumor necrosis factor-α, and lactate dehydrogenase possibly representing a cytokine storm syndrome. Echocardiography may help to assess for global and regional wall motion abnormalities and also demonstrating thickened interventricular septum with associated enlarged left ventricular diastolic diameter, decreased left ventricular ejection fraction, and increased pulmonary arterial pressure. MRI may confirm acute myocarditis and myocardial injury. Endomyocardial biopsy (EMB), long considered the gold standard diagnostic test, can directly demonstrate myocyte necrosis and mononuclear cell infiltrates. EMB will detect evidence of a viral cause in some cases, though in others an immunologically autoimmune-mediated cause of the myocarditis is suspected. However EMB and MRI may be cumbersome and not practical during the current situation but can be considered for research purpose in an appropriate setting.

The various distinct mechanisms for non-ischemic myocardial injuries that have been published in the literature are – [i] inflammation and cytokine storm mediated through pathologic T-cells and monocytes leading to myocarditis and documented by significantly raised inflammatory markers [ii] secondary to hemophagocytic lymphohistiocytosis, [iii] viral myocarditis with reports of progression to fulminant myocarditis, [iv] stress cardiomyopathy, [v] hypercoagulability and development of coronary microvascular thrombosis [vi] respiratory failure and hypoxemia

induced cardiac myocyte apoptosis. COVID-19 induced cardiac injury leads to activation of the innate immune response with release of proinflammatory cytokines. Proteins released through cell lysis like Myosin heavy chain, a cardiac sarcomere protein, appears to be a prime example of ‘molecular mimicry’. Myocarditis appears in COVID-19 patients after a prolonged period (up to 10–15 days) after the onset of infection. Together, the data suggest that a delay in myocardial inflammation is consistent with at least two pathogenic mechanisms: first, that the ‘cytokine storm’ unleashes a subclinical autoimmune myocarditis, and secondly that myocardial damage and/or molecular mimicry initiate a de novo autoimmune reaction.

There is no specific treatment option for myocarditis and need to be managed by supportive therapy like antivirals and early application of interferon, corticosteroids, tocilizumab, anakinra, intravenous immunoglobulin, statin, and active mechanical life support.

Arrhythmias

Viral infections are associated with myocardial inflammation, metabolic imbalances and activation of the sympathetic nervous system, all of which predispose to cardiac arrhythmia. The incidence of arrhythmias has been reported in COVID-19 patients up to 16.7%. The commonest rhythm abnormality noted is tachycardia, uncommonly bradycardia has been noted in some patients. Arrhythmias were observed in 7% of patients without ICU care as compared to 44% of patients admitted to an ICU. A 5.9% incidence of malignant arrhythmias, with a significantly greater incidence in those with elevated troponin level was found (17.3% vs 1.5%, p<0.001). Other arrhythmias seen include atrial fibrillation, conduction block, ventricular tachycardia, and ventricular fibrillation. Others causative factors include dyselectrolytemia, medications affecting QT interval with potential to cause torsades de pointes and fever which may predispose to Brugada syndrome.
and long QT syndrome.\cite{6} Even after hospital discharge, we should consider that myocardial injury might result in atrial or ventricular fibrosis and scarring which may form the substrate for subsequent cardiac arrhythmias. The extent of myocardial scar, as assessed with cardiac magnetic resonance, helps to stratify the arrhythmic risk in patients recovered from COVID-19.\cite{36}

**Acute coronary syndrome**

Acute ST elevation Myocardial Infarction due to plaque rupture or coronary thrombosis due to hyperinflammation may be seen in patients of COVID-19. Patients with risk factors or existing CV disease have a heightened risk of developing an acute coronary syndrome (ACS) during acute infections, including viral illnesses and other acute inflammatory conditions.\cite{32} However the number of patients presenting with Acute MI in Emergency room has been significantly reduced and this has been attributed to the reluctance of patients to go to a hospital during the COVID-19 outbreak, delays in evaluating patients with STEMI after hospital arrival due to precautions such as detailed travel and contact history, symptomatology, and chest X-ray. SCAI and other Expert groups recommend to consider fibrinolytic therapy in select patients with ‘low risk’ ST-elevation MI (STEMI).\cite{37} Additional precautions taken in catheterization laboratory such as time needed to wear protective gear may further delay intervention.\cite{38}

**Heart Failure**

Although data on incidence of left ventricular systolic dysfunction, acute left ventricular failure, and cardiogenic shock is less but available studies have shown heart failure as a complication of COVID-19. ACE2 expression is up-regulated in failing human hearts, which may lead to a higher infectivity of virus and a higher mortality in patients with heart failure. Underlying mechanisms of acute HF in COVID-19 may include acute myocardial ischemia, infarction or inflammation (myocarditis), ARDS, acute kidney injury and hypervolemia, stress-induced cardiomyopathy, myocarditis and tachyarrhythmia. Heart failure has been seen in 23% of COVID patients in a study by Zhen et al. The incidence of significant heart failure in 52% of the non survivors and up to 12% patients who recovered.\cite{18} Also as with any critically ill patients in ICU, they may develop reversible sepsis-related cardiomyopathy with left ventricular dilatation and impaired systolic function. COVID-19 infection can cause decompensation of underlying heart failure and may lead to mixed shock syndrome (combination of septic shock and cardiogenic shock). Significantly elevated BNP/NT-proBNP levels also suggest acute HF.\cite{3} If heart failure is suspected, a limited TTE or focused ECHO can be performed. Invasive hemodynamic monitoring and supportive measures like inotropes and diuresis can be used to treat cardiogenic shock and fluid overload in such cases.

**Venous and Arterial Thromboembolism**

COVID-19 disease causes a prothrombotic state leading to venous and arterial thrombosis. Prolonged immobilization leads to venous stasis and hypercoagulability due to use of glucocorticoid, immunoglobulins as well as vascular endothelial damage due to central venous catheterization and/or ECMO, hypoxia often are a contributing factor for occurrence of VTE. The Exact incidence of thromboembolism is unknown but few case reports have mentioned the occurrence of pulmonary embolism in these patients. Report of occurrence of acute pulmonary embolism in two patients aged 57 and 70 years with elevated D-dimer and multiple filling defects on CT pulmonary angiogram.\cite{39} Multiple studies from China have reported higher D-dimer levels in COVID-19 patients with adverse outcomes.\cite{18} D-dimer levels were significantly higher in non survivors than survivors (2.12 μg/ml vs 0.61 μg/ml; p<0.001) thus reflecting a worse prognosis.\cite{40} Multiple reasons can be postulated for activation of coagulation cascade in critically ill-patients which include i) pro-inflammatory cytokines lead to activation of coagulation cascade; ii) during inflammatory conditions, the alveolar hemostatic balance is tilted more towards a prothrombotic state; iii) proinflammatory cytokines may itself lead to endothelial injury and activation of coagulation cascade. In such a scenario, levels of D-dimer which serves as marker of fibrinolytic activity is elevated along with other inflammatory cytokines. Critically ill patients with COVID-19 are at an increased risk for venous thrombosis and hence the need for anticoagulation in these patients. A recent study showed that in COVID-19 positive patients with sepsis-induced coagulopathy score <4, administration of heparin led to a reduced 28-day mortality. Anticoagulation with heparin has been recommended for patients with raised D Dimer.\cite{40}
**Long term sequel of COVID-19 infection**

Since COVID-19 infection is a new disease it is too early to understand the long-term cardiovascular outcome for patients who have recovered from COVID-19. Patients recovered from previous SARS infections and followed over 12 years showed that 40% had cardiovascular abnormalities, 60% with abnormal glucose metabolism and 68% with hyperlipidemia. [38] Patients recovered from pneumonia followed on long term show cardiovascular abnormalities like myocardial infarction, stroke, and fatal coronary artery disease. These are secondary to increased systemic inflammatory and procoagulant state seen in these patients. The hyperlipidemia may be attributed to the high dose methylprednisolone therapy. In addition, these patients had significantly higher lipid levels as compared to controls which had been attributed to the high-dose pulses of methylprednisolone [41].

**Social effects and consequences on health care**

COVID-19 will lead to a huge burden on developing countries. In a resource limited setup already burdened with existing disease COVID-19 can potentially crash the healthcare system. The focus on COVID-19 treatment has lead to Cardiac diseases taking a back seat. Patients with CVD are not only more susceptible to have COVID-19, the neglect in the care of their primary disease will lead to disastrous consequences. Although control measures like lockdown and curfews are needed due to these restrictions and also fear of contracting disease patients with acute MI delay in coming to hospitals and increasing patients with sequel like LV dysfunction and heart failure are now seen [42]. Hence, strict control measures such as social distancing, lockdown and curfews are the need of the hour to prevent the disease spread. Also, routine follow up and compliance to medications especially in patients from rural areas would be hampered. More emphasis on telemedicine to maintain consultation and follow up with patients and to minimize contact should be encouraged.

**Interventions and cardiac implications**

There are various medications (new and established ones which are repurposed) that are currently being tested in various ongoing studies. Chloroquine and hydroxychloroquine shown promise in some trials when used early in the disease [43]. However clinicians need to be watchful for some rare cardiac arrhythmias associated with these drugs like polymorphic VT (Torsade de Pointes) as they have potential to prolong the QT interval [44]. Convalescent plasma and tocilizumab (a monoclonal antibody against interleukin-6) are other therapies that have shown some promise in reducing severity of illness [45].

Although, ACEi might facilitate viral entry into respiratory cells leading to viral mediated cell damage, these same medications might upregulate ACE2 and reduce the acute lung injury caused by COVID-19. As for now, guidelines recommend that patients on ACEi and ARBs should continue taking their medications as usual and should not discontinue them with COVID-19 disease. Some statins like pitavastatin have shown some effect against COVID but further studies are needed in this regard. [46] NSAIDS have been shown to increase the expression of ACE2 on the cellular membranes and could theoretically increase viral entry into respiratory cells. More studies will be needed on this, but given this concern, many European countries have suggested that in patients with respiratory tract infections, acetaminophen should be used in preference to NSAIDs for control of pain and fever [47].

Remdesivir an antiviral repurposed from previous used for Ebola has shown to be effective in trials to reduce time to clinical improvement and mortality in patients on oxygen [48] but needs larger studies for efficacy and tolerability. Tocilizumab has shown to cause dyslipidemia in patients treated for rheumatoid arthritis. Following tocilizumab, LDL-C, and HDL-C were increased, while cardiac risk markers like phospholipase A2 and Lpa were decreased. [49]
Conclusion

SARS-CoV-2 with its varied presentation and rapid infectivity has emerged to be the biggest challenge faced by mankind in this century. Cardiac patients are at increased risk of severe COVID-19 infection and it is associated with worse prognosis. COVID-19 infection leads to fulminant myocarditis, acute coronary syndromes, heart failure, arrhythmias (Atrial Fibrillation, Ventricular Tachycardia and Fibrillation) etc. all of which are life threatening. Cardiac complications usually appear 10 days after onset of fever and can be detected early using biomarkers. Hence there is need for close monitoring of patients to detect and manage these cardiac complications especially in those with comorbid conditions. Several potential areas of research include pathogenesis and therapeutics; along with randomized control trials are urgently needed which will help in managing this global pandemic.
Ethical Clearance - Review article, no study/intervention on human subjects so ethical clearance not needed.

Source of Funding - Self

Conflict of Interest – nil

Bibliography


Tubo-ovarian Abscess and Uterine Leiomyoma in a 35-year-old woman with 8 years of infertility: A Case Report

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Abstract

Infertility can be influenced by several factors, including the presence of leiomyomas and tubal abnormalities like a tubal abscess or tubo-ovarian abscess which can cause adhesions of the tubes and surrounding tissues or obstruction of the tubes. A 35-year-old woman is diagnosed with primary infertility of 8 years with intramural uterine leiomyoma, right tubo-ovarian abscess, and left tubal abscess with tubal obstruction. Laparotomy myomectomy and salpingostomy surgery were carried out. There were no complications during or after the surgery. The management of infertile patients with leiomyoma and tubo-ovarian abscess who wish to preserve reproductive function requires special attention.

Keywords: Laparotomy, Intramural leiomyoma, Tubo-ovarian abscess, Infertility

Introduction

Infertility is one of the major problems affecting 13-15% of couples worldwide. The causes of infertility in women can be due to ovarian, tubal, peritubal and uterine abnormalities.1 Uterine leiomyoma is the most common benign uterine tumor found in the lower abdomen and affects women in reproductive age. Leiomyomas can be found in 30-40% of all women between the ages of 30 - 40 and are often seen in African women compared to women of other ethnicities.2 Although its association with infertility is still controversial, it is still a major concern for physicians and patients themselves.3 A tubo-ovarian abscess (TOA) is a serious complication of untreated pelvic inflammatory disease (PID). TOA is often found in women of reproductive age and nearly 60% of nulliparous women. Long-term complications of TOA include infertility, increased risk of ectopic pregnancy, and chronic pelvic pain. Since most of these patients are of reproductive age, fertility preservation should be considered when deciding on the optimal treatment strategy.4

Case Report

A 35-year-old P0A0 woman self-referred to the Gynecology clinic in KRMT Wongsonegoro General Hospital Semarang, Indonesia with lower abdominal pain. The pain was most significantly felt during the beginning of her menstruation period for 2-3 days. The patient also felt a mass that has been growing since a year ago. A history of white, odorous vaginal discharge was recognized by the patient since 2 years ago, and only appears a few days before menstruation. Every menstrual period the patient feels abdominal and back pain. The patient admitted that she had never sought medical attention for her complaints before. The patient has been married for 8 years with no children. Currently, the patient is sexually active and does not use any contraceptives.

Assessment of the general condition shows no abnormality. Vital signs were measured: her blood pressure was 123/78 mmHg, heart rate 92 beats/minute, respiratory rate 20 breaths/minute, and 36.9°C body temperature. The patient weighs 61 kg with 155 cm height and BMI 25.3 kg/m², which puts the patient’s
nutritional status in level 1 obesity.

On abdominal physical examination, a mass with the size of a baby’s head was palpated at the umbilical area, mobile, and has a firm consistency. On gynecological examination, toucher vaginal revealed the size of the corpus uteri was as big as an adult fist. Based on ultrasound examination, an intramural uterine leiomyoma was found with a size of 9.4×8.5×6.4 cm and adnexal mass measuring 8.2×9.5×10.2 cm. The abdominal CT scan demonstrated a 9.51×8.46×9.85 cm leiomyoma, as well as multiple cystic lesions with lobulated septa and regular edges at the right adnexa measuring 7.61×9.91×7.9 cm and oval cystic lesions with regular edges margin at the left adnexa measuring 4.27×3.79×3.25 cm. [Fig 1]. Complete blood count showed hemoglobin 13.1 g/dL, leukocytosis (15.5×10^3/µL) and increased neutrophils (75.4%). The remainder of her laboratory results were within physiological parameters.

The patient was diagnosed with intramural leiomyoma, unilateral tubo-ovarian abscess and 8 years of primary infertility. An exploratory laparotomy was performed with an incision on the uterine wall with cautery, an intramural leiomyoma mass with a size of 8×8×7 cm was found, then myomectomy was performed [Fig.3., Fig. 4]. On the right adnexa, the tube was enlarged with the size as large as an adult fist, adhesions to the surrounding tissue were released, and the ruptured tube secreted pus [Fig.2] which gave the impression of a tubo-ovarian abscess, then the abscess was drained salpingostomy was performed. The left adnexal was also enlarged and the ovary tube was widened with tubal obstruction. A salpingostomy was performed. The pelvic and abdominal cavity was washed with normal saline. The operation was completed in 1 hour 8 minutes. The estimated total blood loss was 50 cc. The collected mass was sent for histopathologic examination. There were no complications during and after the surgery. The patient was given a 2 × 1 gr cefotaxime injection afterward. The patient was discharged on the third day in good and healthy condition.
Discussion

The effects of a uterine leiomyoma on fertility are still controversial. The exact mechanism by which leiomyoma affects infertility is uncertain, but the hypothesized mechanisms are as follows (i) blockage of the fallopian tubes; (ii) changes in tubal motility and prevention of fertilization; (iii) prevention of sperm migration through the cervical canal; (iv) dyspareunia; (v) thin and vascular endometrium; and (vi) abnormal uterine movements.

El Mahdi explained that the location and size of the leiomyoma influence on infertility. Intramural leiomyoma and submucosal leiomyoma with intracavity distortion were associated with lower pregnancy rates, implantation, and live birth rates compared with women without leiomyomas. In previous studies, leiomyomas with a size of 2-6 cm did not affect infertility, however, recent studies have shown that intramural leiomyomas > 4 cm in size affect fertility.

Apart from uterine leiomyomas, TOA that occurs in this patient can be one of the factors that cause infertility. TOA is an abscess that involves the fallopian tubes, ovaries, and surrounding pelvic organs. TOA is common in women of reproductive age and is usually a complication of untreated PID or with inadequate treatment. Tubal obstruction due to TOA can inhibit the reproductive process especially the fertilization stage.

Management of TOA and uterine leiomyoma includes exploratory laparotomy, myomectomy, salpingostomy and adhesiolysis. If the patient wishes to preserve her reproductive functions and fertility, myomectomy is the treatment of choice. Pregnancy rates are as high as 50-60% after myomectomy, with favorable obstetric results but postoperative adhesions are of particular concern, as it may harm future fertility.

There are various surgical intervention options for TOA, namely laparoscopic or laparotomy abscess drainage, unilateral or bilateral salpingo-oophorectomy or pelvic clearance. Factors influencing the treatment of choice include previous surgical history, fertility preservation, and the size of the abscess.

In TOA, drainage of the pelvic abscess by irrigation in the abdominal cavity can be considered if fertility is to be maintained. In women with children, salpingooophorectomy can be considered to reduce the chance of recurrence and the need for further surgery. Given that the patient has no children after 8 years of marriage, we did not perform salpingo-oophorectomy and chose salpingostomy to maintain the tube.
Conclusion

A 35-year-old woman with 8 years of infertility was diagnosed with intramural leiomyoma and right tubo-ovarian abscess and left tubal abscess, which is treated successfully with laparotomy, myomectomy and salpingostomy. There were no complications during or after the surgery. The patient was discharged three days after the surgery in good health.

Conflict of Interest: There is no conflict of interest

Source of Funding: Nil

Ethical Clearance: Taken from the Institutional Ethical Committee.

Declaration of Patient Consent

The patient gave their consent for their images and other clinical information to be reported in the journal.

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Prevalence of Drug abuse among ante Partum Females, Obstetric Department, Beni Suef University Hospital, Egypt

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Abstract

The consumption of drugs of abuse among females in the Health Services is a frequent problem, but often it is not diagnosed. Aims: We have analyzed the variations in the clinical profile of female patients came to obstetric emergency department in a period of sex months and made screening for various drugs to assess the percentage of addicted females in this period of life. Secondly, we have assessed also the accuracy of the presumptive method of detection compared to the confirmatory method. Method: Urine samples were taken from pregnant females came to obstetric emergency department, Beni Suef University hospital and was screened to detect the drugs of abuse that taken by these females by immunoassay and GC- mass and clinical assessment of the babies were done. Results: from 300 females there were 20 cases Tramadol positive by immunoassay but all of them were false positive when GC-mass was used. There was a questioned cross reactivity between Ranitidine and tramadol by Immunoassay needing for more researches. 62.3% of the female were no educated 42% of the female’s husbands were SHISHA smokers. Intra uterine growth retardation was the most common fetal complication

Conclusions: scientific survey which done to detect drug abuse among group of people should never depend on immunoassay methods. The belief that women are more biologically complicated than men and that they were too busy caring for their children to participate in studies should be changed. Women should be included in clinical researches.

Keywords: drug abuse, ante partum female, immunoassay, cross reactivity, GC-mass, Beni Suef

Introduction

The problem of drug abuse is not in the individual alone but its general impact on the family and society. The impact of addiction is catastrophic for the family as a whole; Relationships suffer, financial sources get depleted, health costs increase. Also, the consequences of addictive devastating sexual relations and the resulting sexual transmitted diseases and epidemics which is difficult and sometimes impossible to cure them.

Addiction is also strongly associated with domestic violence, which increases the physical and emotional distress of the family. Having an addicted woman in the family is a catastrophe as women are the backbone of the family, the mother and the wife, and women are also the most suffering from men as a result of addiction. Therefore alternative strategies to identify women with problems related to drug abuse should be used.¹
Scientists have discovered that the problem of addiction in women has to do with hormonal changes such as menstruation, pregnancy, childbirth, breastfeeding and menopause. Also, women themselves describe unique reasons completely different from men for using drugs, including get rid of excess weight, fighting exhaustion, coping with pain, and attempts to self-treat mental health problems.\(^2\)

Immunoassays dominate urine drug screens (UDSs) because they are simple to use, easy to automate and provide rapid results. Unfortunately, they are subject to cross-reactivity with structurally related and unrelated compounds potentially yielding false-positive results.\(^3\)

Because many Technicians have limited knowledge of immunoassay cross-reactivity data, patients with false-positive results may be inappropriately terminated from employment or suffer from medical staff bias because of lack of trust and also contribute in giving false survey on the accurate number of drug abuse and gives a false bad impression about a particular sector of population. As a result, programs are put in place to combat addiction in the wrong unsuitable places.

Opiates belong to a large class of compounds characterized by their ability to interact with endogenous opiate receptors. Synthetic opioids as Tramadol that bind to opiate receptors generally require separate immunoassays for screening purposes.\(^4\)

The current study was done on 300 pregnant female in Beni Suef City. Samples were collected from obstetric emergency department, Beni Suef University hospital in a period of 6 months (January to June) in the year of 2019. Every one filled a complete written consent and a questionnaire before collection of samples. Confidentiality of records was kept. The screened drugs were cannabis, benzodiazepine, morphine, tramadol and Amphetamine.

**Samples collection**

Urine samples (usually 10-50 ml from each driver) were collected from drivers in plastic containers, transferred to lab in ice boxes and free zed in \((-20^\circ C)\) until analysis. The samples were collected in front of guardian to avoid dilution of the samples. Each sample took a serial number in view of the females.

**Samples analysis**

**Screening:** All samples were screened by dip stick to detect studied types of drugs at forensic lab at Beni Suef University.

**Confirmatory:** positive samples were confirmed by G.C at forensic lab at Beni Suef University.

**(1) Dip stick**

**Principle of use of dip stick to examine urine sample before extraction:**

It is an immunoassay based on the principle of competitive binding. Drug which present in the urine specimen compete against their respective drug conjugate for binding sites on their specific antibody. During testing, a urine specimen migrates upward by capillary action.
Results

**Negative:** - A colored line in the control line region (C) and a colored line in the test line region (T) for a specific drug indicate a negative result. This indicates that the drug concentration in the urine specimen is below the designated cut-off level for that specific drug. The shade of color in the test region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

**Positive:** - A colored line in the control line region (C) but no line in the test line region (T) for a specific drug indicates a positive result. This indicates that the drug concentration in the urine specimen exceeds the designated cut-off for that specific drug.

**Invalid:** - Control line fails to appear due to insufficient volume or incorrect procedural techniques. These is the most likely reasons for line failure.

(3) **Gas chromatography:**

Instrument: Thermo scientific Trace1300 Gas chromatography

Type of column: Thermo scientific .TG-SQC. Length: 30 m, I.D:0.25mm.

Carrier gas: Helium

Flow rate: 1 ml/ min

Injection volume: 1 micro

Detector: Thermo scientific Trace, ISQ.QD. Single Quadrupole Mass Detector.

Retention time: time of standard peak start from time of injection to time of appearance of peak.

**Method of extraction**

2 ml urine + 5 ml dichloromethane then vortex for 10 minutes then centrifuge. Aqueous part is discarded and solvent is evaporated then reconstitution with 50 micro methanol. Reagents used are G.C grade.

Questionnaire were answered by pregnant female including age, Educational level, Medicinal history, Maternal complication, Premature labor, Fetal complication, Drug intake by Husband.

**Ethical Considerations:** Written consent was taken for all participant pregnant female in line with the Ethics protocol of medical research.

**Results**

About 62% of the participated females were non-educated. Ranitidine was the most common drug (4.7%) which had taken during pregnancy while about 91% of the participated females were not on any drug during pregnancy. 12% of the ante-partum females have pre-eclampsia followed by Diabetes Miletus 3.3% as a maternal complication during pregnancy. 42% of their husband were Shisha smoker, 6.7% were cigarette smoker. Fetal complications were variable as IUGR, macrosomia and congenital anomalies. IUGR came as the major fetal complication by 22%. 15.3% of fetus entered in the incubators because of many complications. From 300 females there were 20 cases Tramadol positive by immunoassay but when GC-mass was used all of them were discovered to be false positive. So in our survey there were no cases of drug abuse among 300 ante partum females came to Obstetric Department, Beni Suef University Hospital.

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</thead>
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<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>300</td>
<td>100.0</td>
<td>100.0</td>
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**Discussion**

Immunoassays were developed for general screening. Urine drug testing is now done for any occupations (i.e., truck drivers and train engineers, any positional governmental employers and also in proposal for many private works). Interpretation of drug test results should be performed by a medical trained and certified technician, who can make result interpretation to understand the pitfalls in interpreting urine drug screen results.\(^7\)

According to the manufacturer reports there are some agents known to cross-react with each class. This list should not be considered the only substances that making the cross react which mean that there are many unlisted substances could make the same cross react. The sensitivity and specificity of the various components of the immunoassays are variable between manufactures.\(^7\)

There are many factors affecting drug detections in urine as UDS’ cutoff or detection level of specified urine metabolite above which the test will be positive, substance’ dose, elimination half-life, urine pH, urine dilution, frequency of use, and time of last use.\(^9\) Thus, health care providers should be aware of all these factors during making their research.

GC/MS is both more sensitive and specific than immunoassay but more expensive, slower, and not readily available for acute management.\(^8\)

Most urine drug screens are designed to detect opiates (namely morphine) and drugs metabolized to morphine by the human body (i.e., heroin) and often “miss” the majority of semi-synthetic and synthetic opioids; although, cross-reactivity does occur and is assay dependent. False positive opiate tests have reportedly been caused by dextromethorphan, diphenhydramine, quinine, quinolones, rifampin, verapamil, and poppy seeds. In the hopes of testing for medical adherence, some urine drug screens specifically include methadone. Of note, false positive methadone screens have been reported to be caused by quetiapine, doxylamine, olanzapine, diphenhydramine, and verapamil.\(^10\)

Many studies reported that, positive screens should be confirmed by GC/MS and / or quantitative levels of the specific drug of abuse in question should be sent in these types of cases.\(^7\)

Immunoassays give to some extent helpful information, but should be treated as ‘presumptive positive’ results which should be confirmed by other an independent procedure such as GC–MS or liquid chromatography–tandem mass spectrometry as we done in our study.

In a study that done on youths and young adults, they suggest that gender may play a role in the patterns of drug use, abuse, and dependence. They found that overall rates of substance use were significantly higher for males than for females.\(^5\) figure 1 highlights country-level variations in the drug use gender gap in Europe.\(^12\) Another data indicate marked differences in the male to female gender ratio in Georgia and Albania.\(^13\)

In our study there was no case of drug abuse among 300 ante partum females while It was estimated that nearly 30 000 pregnant women use opioids in Europe in a survey done in 2009.\(^14\) Also other studies which done in Canada showed that percentage of drug abuse between females and males resemble approximately 1:2.\(^16\)

Our explanation for these results is that Pregnancy and motherhood can be a strong motivator in a woman’s pathway to stop any drug addiction and also sense of guilt and fear of having abnormal children.

Also ethnic, cultural and religious diversity needs to be considered when working with this group of women.\(^15\)

Federal agencies, including the National Institutes of Health (NIH), have been instrumental in pushing for women to be included in clinical research. These efforts have ensured that broader public health issues related to sex and gender are studied.\(^11\)

**Conclusion**

Understanding the reasons for differences between males and females In drug abuse and between females themselves in different countries and continuing to evaluate these patterns over time could help in the development of targeted and more effective prevention and treatment interventions. Scientific survey which done to detect drug abuse among group of people should never depend on immunoassay methods. The belief that women are more biologically complicated than men and that they were too busy caring for their children to participate in studies should be changed. Women should
be included in clinical researches.

**Recommendations**

Because of the poor sensitivity and specificity of immunoassays the clinician usually make definitive confirmation by other methods as GC/MS but it is done in cases with forensic or legal ramifications but this confirmations should be done also in survey studies to give accurate data that can deal correctly through these results. More surveys should be done on bigger sectors of females to detect cases of drug abuse between them.

**Conflict of Interest:** Nil

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Overview of the Content of Bisphenol A in the Amniotic Fluid of Pregnant Women And Its Adverse Health Outcomes

Dardan Dreshaj 1, Flaka Pasha2

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Abstract

Background: This review represents a critical and constructive analysis of literature in the content of Bisphenol A in amniotic fluid of pregnant women and its adverse health outcomes. The review is generated through summary, classification, analysis and comparison of already existing material and researches on field.

Methods: Databases as Scopus, PubMed, Medline, Web of Science, Global Health, were used to extract data for the review. Search terms like “Bisphenol A”, “pregnancy”, “amniotic fluid”, “endocrine disruptors” were used. Out of 200 research articles screened, 70 most relevant studies are included in this review.

Conclusions: This review highlights the correlation between Bisphenol A and its endocrine disrupting potential, impacting especially fragile categories as pregnant women and their fetuses through its toxicokinetic features and its metabolites. Thus, BPA distorts important physiological processes necessary for fetal development and disease progression later in life.

We can conclude that human exposure to BPA, as one of the leading environmental contaminants, represents a major global issue and its adverse health outcomes can be debilitating for human health. Further research on field considering BPA distribution, varying exposure rates, racial disparities, inter-species differences and EDCs cumulative effects, should be conducted. Finding safer alternatives for replacing BPA in market must remain a priority.

Keywords: amniotic fluid; Bisphenol A, pregnancy; adverse health outcomes;

Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl) propane (CAS No. 80-05-7), is a synthetic chemical excessively produced worldwide.1 BPA commercial production began in the United States in 1957, followed by Europe one year later. Its global production grows consistently, varying between 0% and 5% annually, with the strongest growth occurring in China.2 A yearly increase of 4.6% in production of BPA is envisioned to happen from 2013 to 2019.3

Bisphenol A is used to manufacture polycarbonate plastics and epoxy resins.4 Out of the total production of BPA, 65% is dedicated to polycarbonate synthesis, 25% for epoxy resins and the rest 10% for our daily use products such as food storage containers, food antioxidants, metal food cans, baby bottles, thermal papers, dental seals, medical devices, personal care products, safety helmets, sunglasses, lenses, cosmetics, infant incubators, CD/DVDs, hair dryers, fridges, computers and smartphones.5

Bisphenol A is synthetized by the condensation of phenol with acetone in the presence of a catalyst, a strongly acidic ion-exchange resin. BPA consists of a central tetrahedral carbon atom with two methyl and two phenol groups. BPA is relatively water-soluble (120-
300 mg/L),\textsuperscript{6} dissociates in alkaline environment, has a moderate bioaccumulation rate, low vapor pressure, high melting point, and low half-life in air (0.2 days).\textsuperscript{7,8} BPA leaches into environment and impacts human health during its production, processing or waste disposal.\textsuperscript{9} BPA leaching also occurs when polycarbonate and epoxy resin-containing products are exposed to heat, are re-used or their pH changes.\textsuperscript{10} BPA products in contact with heat, acidic or basic conditions accelerate the hydrolysis of the ester bonds between BPA molecules, thus exposing humans to its harmful metabolites. This happens when people heat cans to sterilize food, place acidic or basic food in cans or polycarbonate plastic, and keep heating or washing these products.\textsuperscript{11,12} Even though ingestion of contaminated food is the most common way how human get exposed to BPA, inhalation and skin absorption are considered to be of great importance too.\textsuperscript{13,14} Also, moderate levels of active unconjugated BPA have been detected in human serum, adipose tissue, breast milk, placenta, maternal and fetal plasma, indicating that BPA can accumulate in human body.\textsuperscript{15,16,17}

The United States Environmental Protection Agency (EPA) reported that more than 400,000 kilograms of BPA leach into environment every year,\textsuperscript{18} alarming us on the great burden of exposure, BPA’s cumulative effects and life-long disadvantageous health impact.

**Bisphenol A mechanisms of action and its disrupting potential**

BPA is qualified as a xeno-estrogen that disturbs synthesis, transport, activity and metabolism of endogenous estrogens, consequently affecting the development, growth and reproduction of organisms.\textsuperscript{19,20} BPA can mimic or antagonize endogenous hormones, subsequently perturbing endocrine function, by binding weakly to several steroid receptors including the estrogen receptors (ER $\alpha$ and $\beta$) and thyroid hormone receptor.\textsuperscript{21,22,23} As well BPA strongly binds to transmembrane endoplasmic reticulum, G protein-coupled receptor 30 (GPR30) and estrogen-related receptor gamma (ERR$\gamma$).\textsuperscript{24,25} BPA can also activate peroxisome X receptor (PXR) and the aryl hydrocarbon receptor (AhR), often involved in cross-talk with steroid receptors.\textsuperscript{26,27}

Many of these receptors play an important role in gene regulation, suggesting that BPA may influence normal differentiation and maturation processes especially during embryonic and fetal development.

The United States Environmental Protection Agency established a reference dose (RfD) for humans of 50 $\mu$g BPA/kg body weight (BW) day$^{-1}$, based on a thousand-fold reduction of the lowest observed adverse effect level (LOAEL) of 50 mg kg$^{-1}$ BW day$^{-1}$.\textsuperscript{28,29} Studies indicate that daily human intake of BPA is less than 1 $\mu$g kg$^{-1}$ BW day$^{-1}$, rendering the reference dose to be considered safe to humans.\textsuperscript{30}

However, other studies have shown that administration of low-dose BPA as 0.2 $\mu$g kg$^{-1}$ BW day$^{-1}$ can reduce fertility and sperm production in male animals.\textsuperscript{31,32} At doses of 0.23–23 ng kg$^{-1}$ BPA, the number of ERK-positive cerebellar cells increases and calcium ion signaling in pancreatic cells can be suppressed leading to diabetes mellitus.\textsuperscript{33,34,35}

To add, BPA is thought to elicit aneugenic effects by interfering with microtubule assembly, spindle apparatus function, chromosome segregation during mitosis,\textsuperscript{36,37} and disturbing DNA damage signaling pathways,\textsuperscript{38} thus affecting DNA stability, leading to potential carcinogenesis.

The content of Bisphenol A in amniotic fluid of pregnant women

Human pharmacokinetic data support rapid metabolism of free BPA to its BPA glucuronide (BPAG) and BPA sulfate (BPAS) metabolites, through UDP-glucuronyltransferase (UGT2B15) and sulfotransferase (SULT1A1) enzymes, resulting in faster urinary excretion of BPA in adults.\textsuperscript{39,40}

In comparison to adults, human fetuses and neonates have reduced capacity for chemical detoxification.\textsuperscript{41,42} Studies report that mammalian placenta presents with $\beta$-glucuronidase (GUSB) and steroid sulfatase (STS), breaking down inactive BPA metabolites to free BPA.\textsuperscript{43,44}

Performing liquid chromatography coupled with mass spectrometry (LC/MS) to compare levels of
conjugated and free BPA, in second and third trimester amniotic fluids, detected free BPA levels to comprise of 83% and 91% of total BPA, highlighting the role placental β-glucuronidase has on deconjugating BPA, thus exposing fetuses to even higher amount of free BPA and potentiating its adverse effects.45

A considerate number of studies measured BPA in fetal cord blood 46,47, fetal liver 48,49,50, and amniotic fluids 51,52, on concentrations varying from 0.14 to 9.2, 1.3 to 50.5, and 0.36 to 5.62 ng/g, respectively. Potential correlations between maternal and fetal blood in pregnant women, and between peripheral blood and peritoneal fluid in non-pregnant women, unveiled BPA levels ranging from non-detectable to 4.46 ng/ml for maternal serum (MS) and from non-detectable to 4.60 ng/ml in fetal serum (FS) of pregnant women. In the other hand, BPA levels in non-pregnant woman ranged from 1.30 to 8.17 ng/ml in peripheral blood and from 0.19 to 13.45 ng/ml in peritoneal fluid. Thus, positive correlation between maternal and fetal serum was found, highlighting a continuous distribution of BPA between the mother and fetus. Further, differing BPA concentration levels between pregnant and non-pregnant women reveal the role pregnancy has in underestimating the actual levels of BPA in blood.53

A more complex study, using novel enzyme-linked immunosorbent assay (ELISA), compared BPA concentration on blood samples obtained from healthy premenopausal women, women with early and full-term pregnancy, ovarian follicular fluid, amniotic fluid and umbilical cord blood at full-term delivery.

Surprisingly, results reviled there was 5-fold higher concentration of BPA in amniotic fluid in comparison to other fluids, ranging between 8.3 +/- 8.7 ng/ml at 15-18 weeks of gestation. Findings suggest significant exposure during the prenatal period and accumulation of BPA in fetuses, which must be cautiously considered in evaluating the potential human exposure to endocrine-disrupting chemicals.54

Differently, measuring BPA concentration levels in pregnant Korean women resulted in slightly higher BPA concentrations, ranging from non-detectable to 66.48 microg/L in mother serum, and from non-detectable to 8.86 microg/L in umbilical cords.55 These higher BPA rates in Korean pregnant women may be attributed to different geographical exposure rates to BPA, having in mind that BPA market in Asia raised 13% annually from 2000 to 2006.56 So, different geographical exposure rates, should be considered when interpreting BPA concentration levels.

Moreover, a nested cross-sectional study revealed significant racial disparities in maternal and fetal BPA concentrations. African-Americans had 10-fold higher maternal serum BPA concentrations than Caucasians (30.13 vs 3.14 ng/ml(-1); P=0.038), Hispanics had intermediate concentrations with a trend towards higher concentrations compared with Caucasians (24.46 vs 3.14 ng/ml(-1); P=0.051) and Hispanics had higher fetal BPA concentrations than non-Hispanics (2.05 vs 0.35 ng/ml(-1); P=0.025). These findings potentiate the immediate need to determine if such differences come from different levels of BPA exposure, fetal-placental transfer and its metabolism, or racial genetic variations.57

It is crucial to know that measuring BPA as the end analyte might lead to inaccurate estimates, considering potential interferences from background sources during sample collection and analysis. Aglycone BPA and its primary conjugates as BPAG, BPAS, represent better candidates for biomarkers of BPA exposure, since they are not prone to external contamination and require in vivo metabolism.58 To 2016 only ten studies reported analytical methods to measure BPA metabolites instead of just BPA. Research was limited by either lack of commercial or custom-synthesized BPA conjugates, or lack of labeled internal standards.59,60,61,62,63,64,65,66

In order to achieve even more accurate and comprehensive representation of human exposure to endocrine disrupting chemicals, such as BPA, it is crucial for the future studies to consider cumulative effect of EDCs, knowing that humans are not exposed separately to just one EDC at a point of time.57,68,69

BPA adverse health effects in fetuses and disease progression later in life

Exposure of rodent fetuses to Bisphenol A, at doses similar to environmental exposure, is found to cause postnatal estrogenic effects, as alteration of mammary gland morphology, detrimental long term effects in vagina and faster growth and puberty in females. As
well, reduced sperm production in males, increased prostate weight, and disruption of sexual differentiation in the brain was noticed.\textsuperscript{70,71,72,73,74,75,76}

Short time exposure to so considered “safe levels” of BPA, proved to have direct adverse effect on remodeling uterine spiral arteries, thus limiting blood supply to fetus \textsuperscript{77}, resulting to implantation failure, spontaneous abortion, recurrent miscarriages, or even leading to an increased risk of pre-eclampsia.\textsuperscript{78,79,80,81}

Studies support that women with detectable bisphenol A (BPA) concentrations have significantly higher risk of being infertile. La Rocca et al. \textsuperscript{82} found that the mean concentration of BPA was twice as high in infertile than fertile women (10.6 vs. 4.8 ng ml\(^{-1}\)). Among infertile women, estrogen receptor alpha (ER\(\alpha\)) and beta (ER\(\beta\)), androgen receptor (AR) and pregnane X receptor (PXR) were significantly expressed higher than in fertile patients, highlighting the distorting effect BPA has on these receptors.\textsuperscript{83}

Increased BPA concentrations are also reported to raise the occurrence of polycystic ovary syndrome (PCOS) \textsuperscript{84}, and are related to abnormalities in uterus morphology and endometriosis \textsuperscript{85}. Some other studies prove that BPA may cause atopic hyperplasia, uterine polyps or even cervical sarcoma and nipple adenoma.\textsuperscript{86,87,88}

![Figure 1: Effect of BPA on women reproductive processes. BPA, bisphenol A \textsuperscript{89}](image)

In addition, BPA-treated testes contain mostly spermatogonia and spermatocytes with markedly less round spermatids, indicating signs of meiotic arrest. Neonatal BPA exposure disrupts meiosis during the first phase of spermatogenesis, due to inhibition of BOULE (conserved key regulator for spermatogenesis expression and up-regulation of ER\(\alpha/\beta\) expression in BPA-exposed developing testis) \textsuperscript{90}. This situation leads to increased presence of apoptotic cells in seminiferous tubules, sperm cells DNA damage and decreased sperm counts.\textsuperscript{91}.
Rather than just having disruptive effects in reproductive tract, high BPA concentrations correlate with increased incidence of obesity and earlier puberty in females, altered physical and mental development in children, modified childhood behavior, cardiovascular disease and immune system dysfunction.92,93,94,95

A ten year study, comparing BPA levels in amniotic fluid between pregnant mothers with normal and abnormal karyotype fetuses, highlighted that mothers with abnormal karyotype fetuses had higher levels of BPA concentrations in amniotic fluid, in comparison to pregnant women with normal karyotype fetuses. Therefore, these findings highlight the distorting potential BPA has in DNA stability and carcinogenesis induction.96

Studies aiming to evaluate the role of BPA in carcinogenesis97,98 have indicated that exposure to BPA may increase the incidence of multiple cancers, as breast cancer 99,100,101, ovarian cancer 102,103, uterine cancer 104, prostate cancer 105,106, testicular cancer 107, and liver cancer.108

**Conclusions and Perspectives**

Governmental restriction on BPA and general public concern regarding bisphenols adverse health effects, increased manufacturers interests on developing BPA substitutes such as Bisphenol S (BPS) and Bisphenol F (BPF).109 Due to their stability in sunlight and high temperature, they were initially thought to be safer alternatives.110 In contrast, in vitro studies found that BPS and BPF can elicit even greater estrogenic activities than BPA111, can decrease cell viability, increase DNA damage and induce reproductive and neural toxicity.112,113,114,115,116

In addition, having in mind BPA’s accumulation potential, its distribution to fetal-placental unit, interspecies placental differences117, high BPA exposure in early weeks of gestations, racial disparities, varying geographical exposure rates, and BPA’s cumulative effects with other EDCs, no endocrine disruptor dose should be assumed to be safe in pregnancy.118,119

Therefore, increasing population’s awareness around BPA adverse health effects through health education, and avoiding exposure to products containing BPA, remains the fastest and easiest way to limit BPA long life term adverse health outcomes.

**Ethical Clearance**- Taken from PhD studies committee

**Source of Funding**- Self funding

**Conflict of Interest** - Nil
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Study of Students’ Perception Regarding Open Book Assessment and Closed Book Exams

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Abstract

Background - An “open book examination” is one in which students are allowed to refer to their notes, textbooks, and other approved material while answering questions. This practice is mostly unheard of. It is ideally suited to professional courses like MBBS which especially aim at developing the skills of critical and creative thinking.

Materials and Methods – After a lecture on chapter Medical Law and ethics 100 students selected through simple random sampling after obtaining informed consent were given Pre test. Then they were divided into 2 groups. The topic Medical Law and Ethics was divided into 2 Parts. The students of Group 1 undertook open book exams for Part A and Self study exams for Part B and for students of Group 2 it was reversed. Post test was done with the validated pretest questions. To check the retention another open book exam was performed after a month. The students also answered a questionnaire on their perception of open book exams and the reason for their preferences and it was analyzed.

Conclusion – A total of 100 students participated in the study. In that 60 were medium achievers and 40 were high achievers. Both medium achievers and high achievers obtained high scores in open book exams. Most of the students felt that Open book exam is less time-consuming for preparation, less stressful and increased their Self-directed learning. Open book exams is better to the closed book exams and inculcates the habit of life-long learners.

Introduction

The present time is accepted as the era of advancing knowledge, rapidly changing science and information technology. Consequently there have been many changes in the life styles of people. In yester years the student use to go to the place of Guru, reside there and learn. The test conducted by the “Guru Dronacharya” and the contemporary three hours written test of closed book type have wide difference.1

As per cognitive psychological research, examination or test enhances the retention of knowledge and known as testing effect.2-4 A closed book examination is probably the most common method of student assessment used in all levels of the education system in India.

The continued use of closed book examinations may encourage our students to live in the past rather than the future. Closed book examinations emphasize heavily on low-level skills such as memorization, instead of testing high-level skills such as the abilities to reason, conceptualize and solve problems

The MCI has also changed its curriculum from knowledge based to Competency based curriculum. Students must now move away from passive reading

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of prescribed texts to the process of acquiring skills for lifelong learning. Hence medical educationalists are inspired for the innovation in assessment too. Here is one such attempt to compare the Open Book Exam with traditional Closed Book Exam.

**Materials and Methods**

The present study was conducted at Mysore Medical College and Research Institute, Mysore after obtaining ethical clearance from the Institutional Ethics committee. 100 students were selected through convenient sampling. A written informed consent was obtained from the students.

Didactic lectures will be conducted on the topic “Medical Law and Ethics”. After the lecture a validated Pre test of 200 multiple choice questions was administered to the students.

The second year students were divided into two groups by stratified randomization based on their previous internal assessment score. The students who had scored were divided into high achievers (Group 1) and medium achievers (Group 2) with 60 percentage of marks as the cut off. The topic Medical Law and Ethics was also divided into Part A and Part B.

The students were given a month time. The students undertook an exam in the form of short answers. Group 1 students were administered Open book exam for Part A and Closed book exam for Part B and the order was reversed for Group 2 students.

A post test was done with the same set of 200 Multiple choice questions containing 100 MCQs from Part A and Part B respectively. The gain in the score of open book exam was compared with that of the closed book exam. To assess the retention of the open book exams delayed post test was administered to the students after a month. Feedback was taken from the students on the open book exam through a validated questionnaire in the Likert scale Type (Type 1 – Type 5, Strongly agree to strongly disagree respectively)

**Results**

A total of 100 students were included in the study among them 40 were medium achievers and 60 were high achievers. Perception of MBBS students about the open book exam is depicted in the Table 1. Majority of the students opined that the open book exam was less stressful, less time consuming because of less memorization hence not boring. The majority of students felt that the open book exam assessed their ability to apply knowledge and also inculcated the habit of self directed learning.

Table 2 depicts the comparison of gain in score between open book, closed book and repeat open book test. The high and medium achievers had higher score in open book exam as compared to the closed book exams. In the repeat test too both high and medium achievers showed higher gain scores.

### Table 1 - Perception of students about the open book test.

<table>
<thead>
<tr>
<th>Perception of Open Book Exam</th>
<th>Strongly Agree</th>
<th>Agree</th>
<th>Undecided</th>
<th>Disagree</th>
<th>Strongly Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open book exam is less time-consuming for preparation</td>
<td>52</td>
<td>22</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Open book exam is less stressful</td>
<td>59</td>
<td>27</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Open book exam involves less memorization</td>
<td>36</td>
<td>37</td>
<td>13</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Open book exam has more room for logical thinking</td>
<td>14</td>
<td>27</td>
<td>15</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Open book exam is boring</td>
<td>21</td>
<td>11</td>
<td>42</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>Open book exam assessed the ability to apply knowledge</td>
<td>19</td>
<td>26</td>
<td>27</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Open book exam increased my Self-directed learning</td>
<td>31</td>
<td>27</td>
<td>27</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 2 - Comparison of gain score between self study, open book test and repeat open book test.

<table>
<thead>
<tr>
<th>Students</th>
<th>Median Score achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Closed book test</td>
</tr>
<tr>
<td>Medium achievers</td>
<td>44</td>
</tr>
<tr>
<td>High achievers</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>47.5</td>
</tr>
</tbody>
</table>

Discussion

Since assessment is not unique to any of the specific academic discipline. The closed-book exam is a well-established approach to assessment in higher-education. It is both widely accepted by educators and frequently used, it basically tests how well a student uses the knowledge they can recall with no additional material available for use on the exam. On the other hand, open-book exams allow students to consult textbooks, notes, and other course-related material during the exam. Some educators may consider open-book tests less conventional, but they have gained popularity.

Open book exam is believed to be able to enhance deeper learning among the learners. There is also sufficient evidence to show that open book exam could have a significant contribution to learning at higher taxonomical level. Specifically, students liked the idea of open-book exams, but not necessarily for the reasons educators might think. Anecdotally, students indicated they learned more through the open-book testing approach than they do through the conventional closed-book approach regardless of their grades in the assessments, because they were able to focus on mastering concepts rather than memorizing aspects they could look up in the textbook. Further, they also indicated they did not have a false sense of security going into the open-book exams, as they knew they would have to be able to apply concepts rather than simply report facts from the textbook.

In the present study gain in scores was higher and the retention of the learning was more in both medium and higher achievers. Most of our findings are in agreement with the study done by Pragnesh Parmar except in their study retention for repeat exams was lesser in medium achievers.

In the present study it was observed that the students had appeared for open book exams with all the relevant material necessary. They were even aware as to which topic is better described in which book, they could identify the topic in those books within a few minutes, which was similar to the study conducted by Pragnesh Parmar and Chen at al.

Conclusion

In our study we found a partial support that open-book exams would significantly increase students’ learning, we believe our study was successful in demonstrating usefulness of this type of assessment and further research in this regard.

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Mandibulectomy in Desmoblastic Ameloblastoma: Physiotherapeutic Approach in a Sporadic Oral Surgery Case

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Abstract

Introduction: Ameloblastomas are slow-growing lesion with local infiltrative and odontogenic tumors of epithelial origin. Desmoblastic ameloblastoma is traditionally considered to be a subtype of SMA. The purpose of this case report is to describe the physiotherapy rehabilitation in mandibulectomy of the right side. A patient chief complaint was inability to open mouth, inability to eat food, impaired cognitive and social behavior. The primary goals of mandibular reconstruction are feature restoration and an appropriate cosmetic outcome. The diagnosis of desmoblastic ameloblastoma was confirmed by bone biopsy. Mandibulectomy of right side done due to desmoblastic ameloblastoma. Physiotherapy treatment is found to be best to resolve patient’s chief complaints.

Results: The case report demonstrated that patients with Mandibulectomy with minimal mouth opening regain near-normal range and improvement in social skills.

Conclusion: The patient was able to do essential activities of daily living like eating and talking without pain and regain near-normal face appearance due to physiotherapy intervention.

Key words: Ameoblastoma, Mandibulectomy, Physiotherapy, Rocabado mobilisation, Quality of life.

Introduction

The origin of Ameloblastoma is from the odontogenic epithelium and it is the second most common tumor, only next to odontoma. The tumor is considered benign even with its locally invasive nature (1). Ameloblastomas signify 1 to 3 percent of all oral and maxillomandibular cysts and tumors. The most common type of multicyctic/solid ameloblastoma is 91 percent of all ameloblastoma.

There are several options available for restoring the defects after surgery. The primary goals of mandibular reconstruction are feature restoration and an appropriate cosmetic outcome. Many options like vascularized or non-vascularized bone grafts, bone morphogenic protein, and osteogenesis disturbance (OD) are choices. Desmoblastic Ameloblastoma case mandibulectomy is the surgical option though it is noncarcinogenic (2).

To avoid arbitrary conclusions, a reasonable exercise planning must be proposed by scientific research society (3,4).

Patient information

A case of 51-year-old female severely affected by cystic lesion over the right mandibular body region is presented in the study, wherein the final diagnosis was desmoplastic ameloblastoma of the right mandibular region.
body region (figure 1). The patient complains of painful swelling over the lower right back region of the jaw which was initially small in size and gradually increased to present size 7×4 cm approximately. No history of regression in size of the swelling. History of associated pain which was gradual in onset, dull aching, intermittent, and localized in nature. Pain aggravated on mastication and relieves on its own over a time. There was no history of associated trauma or burning sensation on the consumption of hot and spicy food. Positive history of change in consistency of saliva from normal to thick and ropy since 1 month approximately. Reduced in salivation for 1 month. Composite resection of lesion, segmental mandibulectomy from 41 to angle of mandible of the right side, and reconstruction with peak implant was done (figure 2). Then from next day physiotherapy treatment was started.

Assessment

The patient in this study had difficulty in opening mouth, swelling over the right cheek, difficulty in eating, and talking. The patient was disturbed due to the pain and appearance of the face. Neck movement was normal. Further assessment was taken by FONSECA questionnaire.

Physiotherapy intervention

The patient received physiotherapy for 1 week on a regular basis in the oral surgery ward by a skilled Musculophysiotherapist. Facial muscle exercises and mouth opening actively within the pain limit done by the patient.

The treatment was started with the goal to reduce pain and increase mobility. Further training was carried out when the patient came to the physiotherapy department on a regular basis. Proper mobilization and exercise were given to patient. Joint mobilization can be performed in various directions to improve the joint play at TMJ, such as distraction, distraction given with anterior glide, distraction given with anterior glide, and lateral glide right/left and lateral glide without distraction. And Rocabado’s 6X6 exercise program has various exercises that had to be performed 6 times a day with 6 repetitions of each exercise and it includes rest position of the tongue, shoulder posture correction, stabilization of head flexion, axial extension of the neck and controlled TMJ rotation.
Result

Improvement in range of mouth opening and reduction in pain. Due to early exercise reduction of swelling over face and eating was easier to patient. The patient was depressed by facial appearance and difficulty in talking. After physiotherapy sessions, pain and swelling were reduced so the patient could talk properly and evenness in facial muscles nearly same.

Discussion

Ameloblastomas (odontogenic tumors) of epithelial source and are sluggish rising lesion with home-grown infiltrative development possible (5). Ameloblastomas, excluding odontomas, are the most common odontogenic tumors. Desmoplastic ameloblastoma varies from other predictable ameloblastomas meticulously, in the clinical sense, and X-ray prediction (6). OPG (Orthopantomogram) and FNAC (Fine Needle Aspiration Cytology) were done in the above patient to confirm the diagnosis.

In this case, the patient represented with painful swelling over the lower right back region of the jaw and gradual increase in swelling. After Composite resection of the lesion, segmental mandibulectomy from 41 to angle of mandible of right side and reconstruction with peak implant was done referring to physiotherapy, patient complains of pain and swelling on the operated side. A successful plan of care was made following a clinical evaluation. Goals for rehabilitation were set, starting from mild exercises to strengthening and manipulations to the temporomandibular joint. All the exercises were performed with each one six times a day 6 types of exercises and 6 sets; ice packs were applied around the patient’s right TM joint and cheek to reduce the exercise caused discomfort during the therapy session. There are works of literature that show the effects of temporomandibular joint dysfunction recovery for patients, but research on surgical cases like mandibulectomy with minimal mouth opening regain near-normal range. The patient was able to do essential activities of daily living like eating and talking without pain and regain near-normal face appearance due to physiotherapy intervention.

Conflict of Interest – The authors declare that they have no conflict of interest.

Informed Consent – Written and Oral informed consent was obtained from the participant included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Funding Support – No funding support.

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Pattern of Alleged Homicidal Deaths in and Around Cooch Behar Region

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Abstract

Homicidal deaths are a significant public health problem and affect every society around the world. The incidence of homicide has seen a worldwide upsurge with varying trends across geographical locations. Detailed information on the characteristics of victims of homicide from the Cooch Behar region is limited. This article explores recent trends of homicide in the Cooch Behar region. The study was conducted between June 2019 to May 2020 at the Department of Forensic Medicine and Toxicology, Cooch Behar Government Medical College and Hospital, Cooch Behar. 1133 autopsies were conducted over the time-period with 70 homicidal deaths. Most of the victims were males (70\%) with a male to female ratio of 2.33:1. 34.3\% of victims were between the ages of 21 – 30 years. Most of the victims were laborers (18.6\%) while Hinduism was the most common religion (65.7\%). 06:00 pm to 12:00 midnight was the time when the majority of the incidents took place and 68.5\% of victims were brought dead. Injuries by hard blunt weapons and sharp cutting weapons were responsible for maximum deaths, constituting 21 (30\%) and 15 (21.4\%) cases respectively. Multiple regions of the body were involved in 35.7\% cases followed by head and face region with 34.3\% cases. This study is significant as it aims to identify the profile of risk factors of victims of homicide and suggest new lines of research that would help to reduce the number of homicides.

Keywords: Homicide, Victims, Pattern of injuries, Weapon, Survival period.

Introduction

Homicide is the most nefarious and atrocious crime known to mankind having a grave impact on the psycho-social, political and socio-economic aspects of a country.\textsuperscript{1} The word homicide has originated from two Greek words “homos” which means human beings and “cidos” which means destruction.\textsuperscript{2} It is the intentional act of taking another person’s life and is one of the leading causes of unnatural deaths.\textsuperscript{3} The United Nations Office on Drugs and Crime (UNODC) has declared that homicide is a powerful indicator for determining a country’s level of violence and safety.\textsuperscript{4} In recent years, the incidence of homicide has seen an upsurge throughout the world due to various factors like population explosion, changing lifestyle and exposure to negative experiences in society, cosmopolitanism of urban areas and the psychological burden it brings along. This has been compounded by the improvised and easily obtainable weapons of violence as well as the negative impact of mass media.

According to the National Crime Records Bureau, the total number of murders in India in the year of 2018 was 29,017; and it showed an increase of 1.3\% over 2017 when 28,653 cases were reported.\textsuperscript{5} While the highest numbers of murders among all the states were noted in the state of Uttar Pradesh (4018), 1933 cases were reported from West Bengal.\textsuperscript{5}

The trends of homicide, the profiles of people committing the crime as well as their victims and the methods adopted by the assailants vary in different parts of the world and different parts of the same country. Cooch Behar is an agriculture-based region in the northern parts of West Bengal which shares international
borders with Bangladesh. With the advent of the 21st century, the area has witnessed radical changes along with rapid developments in segments like industries, real estates and IT firms which has led to a change in its demographic profile. And this transition has also changed the crime profile of the area.

There has been no study on the trends and patterns of homicidal deaths in this area till date. Hence the present study was undertaken to analyze the incidence and trends of homicides occurring in the region of Cooch Behar. The insights procured from this study will help to increase the efficiency of criminal investigations and improve the management of human resources and materials besides looking for avenues to curb this menace. We also aim to identify the profile of risk factors of victims of homicide, which may help to prevent and safeguard those at increased risk in the entire society.

**Materials and Methods**

This observational, descriptive, and analytical study was conducted at the Department of Forensic Medicine and Toxicology, Cooch Behar Government Medical College and Hospital. The period of study was from the 1st of June 2019 to 31st of May 2020. All the cases brought to the department for medico-legal autopsy during the specified time-period which were either confirmed by investigating officers before autopsies as homicidal or found to be homicidal at autopsies or declared as homicide during the investigation were taken into account. The unknown and decomposed bodies were excluded from the study.

A detailed history of the cases was initially noted from the inquest reports, bed head tickets, injury report and other relevant documents provided. Other associated information was gathered from the deceased’s close relatives, friends, police and other available persons who were present at the time of the incident and those accompanying the victims, with special reference to General information like Name, Age, Sex Religion and occupation. The suspected patterns of homicide and affected body parts were preliminary noted from the investigating report submitted by the police i.e., the inquest paper. These were further corroborated with the autopsy findings. Details of the crime scene are obtained from crime scene visits or photographs of the crime scenes.

The data collected in a predetermined format during this study was analyzed in tabular form along with its representation in form of diagrams and charts like bar diagram and pie charts etc.

**Results and Analysis**

In the present study carried out at Cooch Behar Government Medical College & Hospital over a period of 12 months, 1133 autopsies were conducted. Out of these, 70 cases (6.17%) were of homicidal deaths.

The study demonstrates a preponderance of male victims 49 (70%) over female victims 21 (30%), with a male to female ratio of 2.33:1. The majority of the victims belonged to the age group of 21 – 30 years, with 24 (34.3%) cases, which was followed by the age group of 31- 40 years, with 12 (17.1%) cases. The least number of cases was observed in the 1st decade as well as the 6th decade with 5.7% cases each. No victim aged more than 70 years was registered. (Table -1).

Most of the victims were Hindu 46 (65.7%), followed by Muslims 17 (24.3%). (Figure -1).

Regarding the occupation of the victims, maximum victims were laborers (18.6%), followed by farmers (17.1%) and businessmen (14.3%). Of the 70 victims, 18.6% were housewives while 8.6% were preschool children and students. (Table -2).

The maximum number of homicides took place in the dark of the night between 06:00 pm to 12:00 midnight with 19 (27.1%) cases. 25.7% of cases were recorded from 12:00 midnight to 06:00 am while another 24.3% of cases between 12:00 noon to 06:00 pm. This suggests that cases of homicide are more prevalent during the night hours when committing the crime and escaping the scene becomes easier. (Table -3).

Out of the 70 cases, 48 (68.5%) were spot dead/brought deaths. 28 cases were hospitalized before death, of them, 14 (20%) died within 24 hours of hospitalization, 6 (8.6%) survived for a week while the rest 2.9% victims died after atleast a week of treatment. (Table -4).

When infliction of fatal injuries in respect to body regions was considered, in most cases the injuries involved more than one body region and due to the extensive combinations, they were grouped into multiple
regions. 35.7% of cases had involvement of multiple body regions closely followed by head and face region alone with 34.3% cases. The neck region was targeted in 12.8% cases while no incidence was recorded where the fatal injury was inflicted over the limbs exclusively. (Table -5).

It was observed that hard and blunt weapon was most commonly used to inflict injuries 21 (30%) cases, followed by sharp cutting weapons 15 (21.4%) cases. In 12.9% of cases both hard and blunt as well as sharp cutting weapons were used. Incidence of firearm use was observed in 8 (11.4%) cases. Asphyxial deaths were seen in 10 (14.3%) cases, with 5.7% of cases of ligature strangulation and 4.3% cases of throttling. In another 5.7% cases the pattern of homicide was due to infliction of fatal burn injuries. (Table -6).

Table 1: Age and Sex wise distribution of Homicidal cases.

<table>
<thead>
<tr>
<th>AGE</th>
<th>MALE</th>
<th></th>
<th>FEMALE</th>
<th></th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Percentage</td>
<td>Cases</td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Less Than 10 Years</td>
<td>3</td>
<td>6.1</td>
<td>1</td>
<td>4.8</td>
<td>4</td>
</tr>
<tr>
<td>11 – 20 Years</td>
<td>4</td>
<td>8.2</td>
<td>4</td>
<td>19.0</td>
<td>8</td>
</tr>
<tr>
<td>21 – 30 Years</td>
<td>16</td>
<td>32.7</td>
<td>8</td>
<td>38.1</td>
<td>24</td>
</tr>
<tr>
<td>31 – 40 Years</td>
<td>10</td>
<td>20.4</td>
<td>2</td>
<td>9.5</td>
<td>12</td>
</tr>
<tr>
<td>41 – 50 Years</td>
<td>5</td>
<td>10.2</td>
<td>2</td>
<td>9.5</td>
<td>7</td>
</tr>
<tr>
<td>51 – 60 Years</td>
<td>8</td>
<td>16.3</td>
<td>3</td>
<td>14.3</td>
<td>11</td>
</tr>
<tr>
<td>61 – 70 Years</td>
<td>3</td>
<td>6.1</td>
<td>1</td>
<td>4.8</td>
<td>4</td>
</tr>
<tr>
<td>More Than 70 Years</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>49</td>
<td>100</td>
<td>21</td>
<td>100</td>
<td>70</td>
</tr>
</tbody>
</table>

Figure 1: Religion wise distribution of Homicidal cases.
Table 2: Occupation wise distribution of Homicidal cases.

<table>
<thead>
<tr>
<th>OCCUPATION</th>
<th>NUMBER OF CASES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmer</td>
<td>12</td>
<td>17.1</td>
</tr>
<tr>
<td>Labourer</td>
<td>13</td>
<td>18.6</td>
</tr>
<tr>
<td>Service Men</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Businessmen</td>
<td>10</td>
<td>14.3</td>
</tr>
<tr>
<td>Student</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>Housewife</td>
<td>13</td>
<td>18.6</td>
</tr>
<tr>
<td>Unemployed</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>4.2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>70</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Distribution of cases based on time of incidence.

<table>
<thead>
<tr>
<th>TIME OF INCIDENCE</th>
<th>NUMBER OF CASES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00 Midnight - 06:00 AM</td>
<td>18</td>
<td>25.7</td>
</tr>
<tr>
<td>06:00 AM - 12:00 Noon</td>
<td>10</td>
<td>14.3</td>
</tr>
<tr>
<td>12:00 Noon - 06:00 PM</td>
<td>17</td>
<td>24.3</td>
</tr>
<tr>
<td>06:00 PM - 12:00 Midnight</td>
<td>19</td>
<td>27.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>70</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4: Distribution of cases based on period of survival.

<table>
<thead>
<tr>
<th>PERIOD OF SURVIVAL</th>
<th>NUMBER OF CASES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brought Dead</td>
<td>48</td>
<td>68.5</td>
</tr>
<tr>
<td>Within 24 Hours</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>1 Day To 7 Days</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>More Than 7 Days</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>70</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 5: Distribution of cases based on region of body bearing fatal injuries.

<table>
<thead>
<tr>
<th>AFFECTED BODY PARTS</th>
<th>NUMBER OF CASES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head And Face</td>
<td>24</td>
<td>34.3</td>
</tr>
<tr>
<td>Neck</td>
<td>9</td>
<td>12.8</td>
</tr>
<tr>
<td>Thorax</td>
<td>5</td>
<td>7.2</td>
</tr>
<tr>
<td>Abdomen</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Limbs</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple Regions</td>
<td>25</td>
<td>35.7</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>70</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 6: Distribution of cases based on methods and weapons used to inflict fatal injuries.

<table>
<thead>
<tr>
<th>METHOD AND WEAPON</th>
<th>NUMBER OF CASES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical Injuries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharp Edged</td>
<td>15</td>
<td>21.4</td>
</tr>
<tr>
<td>Hard Blunt</td>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>Sharp Edged + Hard Blunt</td>
<td>9</td>
<td>12.9</td>
</tr>
<tr>
<td>Firearm</td>
<td>8</td>
<td>11.4</td>
</tr>
<tr>
<td>Asphyxial Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ligature Strangulation</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Throttling</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>Smothering</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Drowning</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burns</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Poisoning</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>70</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Discussion

Incidence:

The incidence of homicide in the Cooch Behar region over the time-period of the study was 6.17%. This is in accordance with studies by Gupta et al\textsuperscript{6} - 5.9% at Delhi, Mohanty et al\textsuperscript{7} - 6.9% at Behrampur city and Patowary\textsuperscript{8} - 7.36 at Guwahati region. However, the findings are in contrast with studies by Jhaveri et al\textsuperscript{9} - 2.31% in the city of Surat and Parmar et al\textsuperscript{10} - 2.4% in Bhavanagar city. This difference in the incidence of homicide may be due to the geographical variations and the divergent demographic profile of the regions in which the studies were conducted.

Age and Sex Distribution of cases:

Our study reveals that the maximum number of victims of homicide were in the age group of 21 – 30 years (34.3%) followed by 31 – 40 years (17.1%). These findings are in unison with previous works done by fellow Indian researchers Gupta et al\textsuperscript{6}, Patowary\textsuperscript{8}, Jhaveri et al\textsuperscript{9} and Parmar et al\textsuperscript{10}. This is also concurrent with foreign studies by Hilal et al\textsuperscript{11} and Al-Azad MAS et al\textsuperscript{12} who have also reported that the maximum numbers of victims of homicide are in the 3rd decade of life. But it differs from the studies made by Kominato et al\textsuperscript{13} and by Saint Martin P et al\textsuperscript{14} where those in the 5th and 6th decade of life are more vulnerable. This might be explained by the social structure in their setup. The high incidence of cases in the age group of 21 – 30 years may be since this is the time when the responsibilities of earning and stabilizing in life start growing in the Indian individuals as they escape from the parental society. This added responsibilities, failures in some cases and ever-growing frustration in the fast-paced life lead to frustrations, intake of alcohol and bad accomplices and thereby making the youth susceptible to violence. Also, people in this age group are more combative and less humane, leading to altercations and brawls and ultimately ending in homicides.

Among the female victims, most cases were seen in the age group of 21 – 30 years followed by 11 – 20 years. The prevalence of incidence of homicide among females in this age group is owing to marital and romantic discords. Also, newly married females belonging to this age group are more likely to become victims of dowry death.

Males predominate in all the age groups except in the age group of 11 – 20 years where the ratio was 1:1. The overall ratio of all age groups was 2.33:1. This is in accordance with studies by Buchade et al\textsuperscript{15} (2.23:1), Parmar et al\textsuperscript{10} (2.47:1) and Hugar et al\textsuperscript{16} (2.56:1). The male: female ratio was found to be significantly higher in studies by Patowary\textsuperscript{8} (8:1), Jhaveri et al\textsuperscript{9} (6.57:1) and Shah et al\textsuperscript{17} (3.71:1). A predominance of males victims was also seen in studies abroad as reported by Hilal et al\textsuperscript{11}, Al-Azad MAS et al\textsuperscript{12} and Saint Martin P et al\textsuperscript{14} while Kominato et al\textsuperscript{13} noted a male to female ratio of 1:1. The male predominance may be because they are significantly involved in outdoor activities and are generally more aggressive by nature which makes them more vulnerable to be involved in violent crimes. On the other hand, the low incidence among females may be credited to the social norms, traditions and predilection to stay indoors.

Religion of victims:

The present study revealed that the maximum number of victims belonged to the Hindu community which was in accordance with the findings of other studies from various regions of India.\textsuperscript{7} The reason behind this is the Hindu dominant population in the Cooch Behar region.

Occupation of victims:

Our study finds that most of the victims were laborers (18.6%) and farmers (17.1%) besides 18.6% housewives among females. In a study by Shah et al, maximum victims were laborers (20%) followed by businessman (15%); cultivators or farmers (14%) and housewives (14%).\textsuperscript{17} Similarly, Mohanty et al concluded that most victims were laborers or farmers.\textsuperscript{7} This trend is even seen in a study at Malaysia which revealed that about 72% of the victims were semiskilled and unskilled workers.\textsuperscript{18}

Time of Incidence:

In our study, it was observed that 27.1% cases of homicide took place in the evening hours (06:00 pm – 12 midnight) while another 25.7% cases took place in the late-night (12 midnight – 06:00 am). It was concluded that cases of homicide are more common during the night hours. This may be because a chance of being
recognized reduces in the dark of the night due to lack of public presence, besides the abuse of alcohol and other substances increases considerably at night. This finding is in accordance with studies by Gupta et al, Mohanty et al and Shah et al. But the findings were not consistent with those of Bhupinder et al and Vougiouklakis T. both of whom have concluded that the maximum number of incidences occurred during the day time.

Period of Survival:

Most of the cases were reported as brought dead (68.5%) while 20% of cases died within 24 hours of admission. Even with advancements in life support systems, a mere 2.9% of the cases survived for more than a week. This signifies the use of improvised and lethal weapons, multiple injuries to the vital organs of the body or involvement of more than one assailant in some cases. Our findings are concurrent with Kohli et al who revealed that 60.4% of the victims were brought dead and Shah et al (74% spot/brought dead). Hugar et al, Jhaveri et al and Bhupinder et al all revealed that more than 80% of cases were brought dead which are much higher than our numbers suggest.

Distribution of Injuries with respect to body region:

From our study, it was revealed that the majority of the fatal injuries were over multiple regions of the body (35.7%) closely followed by head and face injuries (34.3%). Injuries to the thoracic region were relatively less at 7.2% which is in contrast to studies by Kohli et al and Marri et al. Marri et al suggested that chest was the primarily targeted area of the body as almost 86% of the cases were due to firearm injuries. Our study is in unison with studies by Mohanty et al and Jhaveri et al both of whom have suggested maximum involvement of multiple regions of the body. Parmar et al and Buchade et al have however concluded that head and neck are the most commonly involved regions of the body. This involvement of multiple body regions may be due to the assailant wanting to ensure that the victim will not recover from the trauma in any way possible or may be due to over-kill or involvement of multiple assailants. Homicidal burn and charring injuries also lead to the involvement of multiple body regions.

Method and Weapon used to inflict fatal injuries:

Most of the victims had mechanical injuries over their bodies leading to death. And most of these injuries were caused by hard blunt weapons (30%), followed by sharp-edged weapons (21.4%). This finding is in accordance with studies by Buchade et al and Singh et al. Similar findings were revealed by Kominato et al at Japan and Bhupinder et al at Malaysia. On the other hand Parmar et al, Hugar et al and Shah et al suggested sharp cutting weapons to be the commonest type of weapons used in the homicide. Cooch Behar is basically an agricultural area with forests in and around. This socio-cultural aspect of the population and the geographical location can be the reason for more use of hard blunt weapons as homicidal weapons. Also use of only hard blunt weapons is a sign of spontaneous and unplanned explosive behavior of the assailant. However, quite contradictory to this, Hilal et al, Saint Martin P et al and Marri et al observed firearms as the most commonly used weapons in homicide. The greater accessibility of licensed and country-made firearms in their respective study areas can be an explanation for such a finding. Overall, the weapon of homicide depends on the socio-economic status of the population as well as the political scenario of that study area.

Our study revealed asphyxia was reported in 14.3% cases with 5.7% cases of ligature strangulation and 4.3% throttling. Shah et al found 4% cases of ligature strangulation and throttling each while Hugar et al revealed 7.0% cases of ligature strangulation and 1.8% cases of throttling. On the contrary Jhaveri et al reported 19.81% of cases of asphyxial deaths which were higher than our number. In our 80% of the victims of asphyxial deaths were either females or children. This is because they offer less resistance to the acts of throttling or strangulation. Buchade et al in their study also concluded that most of the victims of asphyxial deaths are females and children.

Conclusion

Homicide is a complex criminal phenomenon with varying trends over geographical locations. Our analysis of autopsied victims of alleged homicide is an attempt to understand certain aspects of victimology. This understanding is crucial during criminal investigations, since these socio-demographic characteristics offer
information on potential victims, help understand the probable modus operandi of the perpetrators and suggest new lines of research that would help reduce the number of homicides. The facts that have surfaced from our study suggest that to curb this peril the Government should take measures to reduce the unemployment rate and address the issues of marital disputes by enhancing an individual’s social and emotional skills along with psychological counseling. Strict laws on possession of dangerous weapons and firearms should be enforced. Further well designed and large scale multivariate studies need to be carried out to get a clear insight into the psychopathology of the assailants and formulate strategies that can thwart such unlawful human killings.

Source of support: Nil

Conflict of Interest: None.

Ethical Clearance: Taken from institutional ethics committee

References


Evaluation of Posture and Quality of Life in Females Undergone Modified Radical Mastectomy: A Research Protocol

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Abstract

Modified radical mastectomy performed for CA breast is a life modifying surgery for most women. It creates a profound impact on the quality of life enjoyed by the women. Body image in patients with breast cancer differs from all other cancers. The assessment of quality of life after modified radical mastectomy for early breast cancer patients using the World Health Organization Quality of Life-Brief Questionnaire (WHOQOL-BREF).

Women suffers from day to day activities. Postures become impaired. Postural changes, such as increase in thoracic kyphosis, scoliosis leads to poor postural habits. There is also decrease in shoulder range of motion, scapular muscles weakness. Contractures may develop in axilla after surgery. Breast cancer survivors have muscle weakness of upper limb are Pectoralis major, serratus anterior, upper trapezius, rhomboid muscles, latissimus dorsi.

Spine alignment also may effect due to poor posture. Women with Modified Radical mastectomy may have some social phobia. Anxiety, stress level may increase. Women may have fear of recurrence. Because of this factors, there is impaired Quality of Life in Women.

Conclusion: Breast cancer is a serious disease that reduces the quality of life, particularly in physical domain and in the emotional area. Oncological disease is becoming a disease for a lifetime. It connected with the constant fear of cancer recurrence and the consequences of treatment.

Key Words - Posture, Quality of Life, Modified Radical Mastectomy.

Introduction

¹In India breast cancer is most commonly diagnosed as cancer. ²Among women it is most common neoplasm. ³Modified Radical Mastectomy is very common operation in women which causes different changes in the body. ⁴It’s stres ful event in women’s lives. ⁵The life expectancy of breast cancer is longest in categories cancer, the Quality Of Life in patients is most relevant factor to be considered for women undergone MRM.

Problems following surgery. – which may affect female’s working capacity, also her family life and her social involvement. ²After MRM there are various factors like tightness in pectoral region, post surgery contractures in axilla, weakness of scapular muscles and restricted ROM of shoulder will affect body posture. Muscle contractures in the cervical and easy fatiguability scapular region are evident observed. Women suffer difficulty in performing daily activities because of impaired the upper extremity function and some bposture disorders. Postural defects like scoliosis, kyphosis due to trunk asymmetry can lead to poor postural habits.

⁶Improper body posture may cause somatic abnormalities. In Mastectomy, patient’s both physical and psychological aspects should be considered. Complications associated with post mastectomy are changes in body posture caused by disorders in static and dynamic postural changes affecting the appearance thus affecting the ability to function in an ideal way.
restricting women from participating in various activities and thus affecting her QOL. 7 After treatment, women experiences some changes like limitation in movement and postural asymmetry. 8 This is associated with lot of changes in musculoskeletal functions. Because they are abnormal in the development of anteroposterior curve, their position may be incorrect due to functional changes.

9 Recent article suggested that breast cancer surgery can affect posture, spine alignment and increase thoracic kyphosis and decrease shoulder range of motion. Women with modified radical mastectomy constantly fear recurrence of disease. 4 This anxiety in breast cancer patients affects their way of living and further postural changes and difficulty in functioning according to desired patterns may contribute towards reduced quality of life. 10 Quality of Life in women is highly impaired after diagnosis because of the effects of physical, social and psychological factors.

Materials and Methods
Study Setting: Physiotherapy OPD and AVBRH
Study design: Observational Study
Selection criteria:
Inclusion criteria:
1. Females upto 6 months post modified radical mastectomy surgery.
2. Age between 40-70 years.
3. Subjects willing to participate voluntarily.
Exclusion criteria:
1. Females upto 6 months post modified radical mastectomy surgery.
2. Age between 40-70 years.
3. Subjects willing to participate voluntarily.
Data Source :
For Quality of life – self reported questionnaire score will be measured and analysed.
For Posture – Deviation in posture will be measured using Kinect Azure.
Bias : Subjects that does not match the selection criteria will be excluded to prevent bias
Study size: 35 sample size
Quantitative Variables: Score of Quality Of Life.
Statistical Methods: This will be done by using descriptive and inferencive statistics using chi square test students unpaired t test. Software used in the analysis will be SPSS 24.0 version, praphpad prism 7.0 version and p <0.005 is considered as level of significance (p>0.005m)

Findings
After successful completion of the study it will provide evidence on Posture and Quality of life in Females undergone Modified Radical Mastectomy.

Discussion
6,9 The current study is carried out to find out association of postural with Quality of life in females undergone Modified Radical Mastectomy various studies conducted have shown postural deviation in females with breast cancer also that their Quality of life is altered resulting in various difficulties. 4 Current study will be carried to find out whether there is any correlation between posture and Quality of life because changes in posture and spine may affect the working of women whether it may be at work or in household activities because of muscle tightness or other musculoskeletal changes causing restriction at joint decreasing ROM and pain preventing the female to carry out various activities efficiently which may ultimately affect Quality of life. Thus current study aims to find out association between posture and Quality of life.

Limitation : Only Evaluation of posture is done and its effect on Quality Of Life is assessed but not on the treatment outcomes of Modified Radical Mastectomy.

Conclusion
Conclusion will be discussed once study is completed.

Conflict of Interest: There is no conflict of interest.
Source of Funding – No funding needed.

Ethical Clearance: Being a observational study institutional ethical committee permission was taken.

References


Retraction in Orthodontics – A Short Review

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Abstract

Aim- The aim of this review is to discuss about the retraction mechanism in orthodontics and to discuss in detail regarding the mechanism of action and their application in orthodontics.

Objective- To list out the difference types of retraction mechanics used and their merits and demerits

Background- The basic principle of retraction mechanism can be used in orthodontic space closure involving retracing the canine initially followed by remaining anterior teeth or entire anterior teeth can be retracted at once using intra or extra oral anchorage. They are broadly classified into friction and frictionless mechanism. Once the extraction of the teeth has been done, the orthodontist must choose the procedure to retract the teeth based on the demand of the case. This article provides the basic information of both the mechanics and their application in orthodontics.

Reason- This review mainly done for better assessment and benefits of retraction mechanics and its appliances

Keywords- Two step retraction, loop mechanics, enmass retraction, space closure

Introduction

The different methods to close spaces, reduce procumbency, overjet, and eliminate extraction sites by antero-posterior therapy is generally categorized as Retraction mechanics. Whether retracting the anterior or protracting the posterior or a combination of both principles of retraction mechanics apply for space closure remains the same.

As extraction was done in late 19th century finger springs or other methods were employed for simple pushing back of canine teeth. This often resulted in tipping and elongation of teeth. Tweed used the molars as anchorage by tying back the arch and using a coil spring arch to retract the cuspids back with the help of 0.016 round archwire. In 1980, rickets employed closed coil springs to obtain sectional cuspid retraction. Several studies have been made on the amount of force required for specific tooth movements. Storey and Smith’ reported that 150 to 200 grams is the optimum force range for retraction of the lower canine tooth. Reitan’ has reported that the maximum force needed for continuous bodily movement of canine teeth is 250 grams. Burstone and Groves found the optimum force for the retraction of anterior teeth to be 50 to 75 grams. Lee in 1966 showed optimal force for distal movement of maxillary canine with tipping was 15 to 260 cm. Burstone in 1982 developed composite TMA spring for canine retraction. Poul gjessing in 1985 developed a sectional arch technique that produces optimal force system for controlled canine retraction.

Determining the anchorage value while retracting the teeth is of major concern no matter the technique used.

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Maximum anchorage is indicated when only anterior teeth are retracted. Minimum anchorage is used if only posterior teeth is protracted and when combination of both is used moderate anchorage is used.

Retraction is the most frequently used technique in space closure. The strategy used in retraction mechanics must be based on a careful diagnosis and treatment plan made according to the specific needs of the individual. Two step retraction and en mass retraction are two most used mechanics in anterior retraction. In two step retraction retraction of canine teeth is done followed by retraction of all four incisors and en mass retraction involves retraction of all six teeth. Two step retraction involves retraction of canine teeth in one step later retracting followed by retracting the remaining teeth in next step. This will reduce the tendency of anchorage loss by incorporating more teeth in the anchorage unit. However, this will take longer time to complete the treatment. In addition when the entire anterior unit is retracted compared to canine retraction they tend to tip and rotate less, thus requiring less time and effort when compared to canine retraction. En mass retraction occurs when incisors and canines are retracted together. In clinical situation it is difficult to maintain anchorage in clinical situation. Anchorage can be derived from segment of the teeth or the entire arch. In some difficult situations the requirement for extra oral anchorage such as headgear, face mask, intermaxillary elastics and mini implants is needed.

The principles for retraction currently used can be explained as either frictional system in which teeth is allowed to slide distally and it’s guided by continuous arch wire or a non-frictional system with couples and force built into the loops of an arch section.

Mechanics of Tooth Movement

Every free body or an object can be perfectly balanced on one point which is known as centre of gravity. The relationship between line of action of force to the center of the body determines the movement of the free body. In a tooth which is a restrained body, a point similar to the centre of gravity is used which is known as centre of resistance.

If the force passes through the center of resistance an object undergoes translation. The centre of resistance is on the long axis of the tooth for single rooted tooth and probably between one third and one half of the root length apical to alveolar crest for a multirooted teeth.

A body appears to rotate around its center of rotation. The more translational the moment of the tooth the farther apically the centre of rotation would be placed. Moment is defined as tendency to rotate. If the line of action of force does not pass through centre of resistance it will produce some rotation which can be calculated as force multiplied by perpendicular distance from centre of resistance to point of application of force. When two forces of equal magnitude act in opposite direction it will produce a couple. This will produce pure rotation, spinning the object around its centre of resistance. The way an object is rotates while its being moved can be changed by different combination of force and couple.

The ratio between magnitude of couple and the amount of force applied at the bracket determines the type of tooth movement exhibited by the tooth. A single force produces a uncontrolled tipping so in order to produce a controlled tipping or translation a single force is insufficient, a rotational tendency (moment) must also be applied at the bracket.

A force of 100 grams produced at a distance of 10mm from the centre of resistance produces a clockwise moment of 1000gm-mm which will cause tipping of the tooth. we must generate counter balancing moment of 1000 grams-mm so that bodily moment can be achieved since the tipping is undesirable. This can be done by twisting the anterior segment of rectangular wire and fitting it into a rectangular slot. After the wire is engaged in a bracket slot it generates an inherent moment of couple which is a couple produced within the wire itself and now it will result in bodily movement of the teeth.

RETRACTION MECHANICS IN EDGewise

Once the extraction of the teeth is done orthodontist have to plan how to close the space. There are two schools of thoughts of retraction mechanism:

i. Two step canine retraction (Friction or frictionless mechanics)

ii. En-mass retraction (Friction or frictionless mechanics)
FRICITION MECHANICS

A tooth can be moved bodily only when force is applied such that it can pass through center of resistance. When a bracket is placed on a tooth and the force is applied at it, both force and moment is experienced by the tooth. The tooth moves in two planes due to this moment of force. The canine moves mesial out as force is applied buccal to center of resistance due to one moment. The second moment produces distal tipping of tooth is caused because force is applied occlusal to centre of resistance. A moment in opposite direction is produced due to interaction between the bracket and wire which counteracts this moment. When the tooth tips in distally it glides along the archwire till binding occur between the archwire and the bracket. This produces a couple at the bracket which results in distal root moment and hence uprighting of the tooth. As it uprights the moment decreases until the wire can no longer bind. Then the canine retracts along the archwire till distal crown tipping again causes binding. Until the force gets depleted this continuous to take place which is known as walking of canine because of initial tipping of crown followed by root uprighting. The major advantage of friction mechanism is it provides comfort to the patients and less time consuming as complicated wire bending is not required.

V – BEND SLIDING MECHANICS

This was developed by Thomas F Mulligan, this approach is used for closing space by moving each teeth (canine retraction or molar protraction). He gave the concept of differential torque as a means of effective intraoral anchorage. It is obtained by applying unequal alpha and beta moments. An off center V- bend is used in a wire to create unequal moments with higher moment applied to the anchorage teeth. The closer the bend is to the bracket shorter the wire and shorter wire has a higher bending moment than a longer wire. Therefore, a higher moment acts on the bracket which is closer to the V bend than the more distant bracket.

In case of canine retraction using the V-bent mechanics the tooth located closure to the bend is the anchor side and the opposite is the non-anchor side. As the cuspids continue to move distally, the bend is centered and the differential torque begins to gradually disappear. Root parallelism begins to effect as the bend approaches the center.

METHODS OF CANINE RETRACTION IN SLIDING MECHANICS:

1. Elastic module with ligature
2. Elastomeric chain or power chain
3. Intra or inter maxillary elastics to kobayashi ligature
4. Coil springs (stainless steel or NiTi)
5. J-hook head gear
6. Sliding jig and traction
7. Mulligans v bend sliding mechanics

ELASTIC MODULE WITH LIGATURE

This method of retraction has been popularised by Bennett and McLaughlin (Fig A). A single elastic module is used to secure the arch wires to brackets which is attached to canine by a ligature wire extending from the molar. These elastic tie backs are activated 2-3 mm or to twice then original size to generate approximately 100 – 150 grams.

Figure A: Elastic modules

ELASTOMERIC CHAIN OR POWER CHAIN

E chain were introduced into the dental profession in 1960 for canine retraction, diastema closure, rotation correction and arch constriction. (Fig B) They are available in configuration of closed loop, short filament, and long filament chains. It has many advantages such as being inexpensive, relatively hygienic and can be easily applied without arch wire removal and do not depend on patient cooperation. Most of elastomeric chains lose 50% - 70% of their initial force during the 1st day of force application and at three weeks retain only 30% -
40% of their original force. Elastomeric chains should be changed every 4 – 6 weeks 10.

It can be used for distal movement of canines without causing loss of posterior anchorage. It involves the use of headgear with j hooks where the hooks attach along a continuous arch wire mesial to the canines and exert a force over them so that they will slide along the arch wire. Since it incorporated extra oral anchorage in canine retraction, it should be effective in maximum anchorage cases 11.

SLIDING JIG AND TRACTION

0.022 round wire or 0.017 x 0.022 rectangular wires are used in making this jig and it is slide on to the arch wire in addition to a short piece of open coil spring of about 4mm in length. The coil spring lies in contact with mesial end of canine bracket and circle of jig lies on mesial end of coil spring. The traction can be applied to the jig either intra or inter maxillary elastics or by extra oral traction.

MULLIGANS V BEND SLIDING MECHANICS

It was introduced by mulligan 1970’s. The basic principle was to apply differential moments to the teeth via bends in the continuous arch wire while force for retraction was applied by auxiliaries like E chain, coil springs etc. during cupid retraction 12. The 45 degrees v bend are added to the wire and 200 grams of force are applied without removing the archwire using calibrated optic pliers.

The v bend helps in differential medio distal movements on the canines and molars. If the bend is placed off center it creates a short and a long segment. The shorter segment is more rigid and hence applies greater moments. So, if maximal canine retraction is required the bend is placed very close to molar and bicuspid. This causes a strong distal crown moment on the molar which counteracts the auxiliary force tending to move the molar crown forward.

EN-MASS RETRACTION

It literally means retracting group of teeth together as a single unit. The anterior teeth are intruded and retracted simultaneously and also maintaining the torque control however, demand on the anchorage should be evaluated carefully 13. It can be effectively employed in moderate and minimum anchorage cases. In frictionless mechanics retraction is done with a continuous wire with
one closing loop each side distal to cupid. Various loop design are available for retraction and all are having pre-determined vertical heights ranging from 7-10 mm in vertical direction to keep it closer to centre of resistance of tooth.

In 1990s, a method of controlled space closure was described using sliding mechanics. Rectangular archwire 0.019 x 0.025 wires are recommended with the 0.022 slot. This wire size had good overbite control while allowing free sliding through the buccal segment. Thicker wires sometimes restrict free sliding of molars and premolars and thinner wires have less control. Thinner wires along with the heavy forces of E-chain can give rise to roller coaster kind of effect.

Soldered hooks of 0.7 brass hooks can be used and alternatively soft stainless steel (SS) 0.6 soldered hooks can also be used. The most common hook length are 36 – 38 mm in upper and 26 mm in the lower arch.

Active tiebacks refer to use of stainless-steel ligatures threaded through an elastic module that goes directly from the terminal molar to the canine bracket. They are stretched to their original size during activation, without pre stretching the force levels range between 200-300 grams. If large spaces are to be closed Niti coil spring are used instead of elastomeric module. The force decay in the Niti coil spring is very much less as compared to elastomeric modules.

FRICTION ISSUE IN SLIDING MECHANICS

Using the friction mechanism has few disadvantages during retraction because of mainly two components friction and binding, due to which applied force should be higher than the required optimum force because of decay in force. When E chain is used for retraction if excessively stretched leads to breakdown of internal bond leading to permanent deformation. It also absorbs water and saliva when exposed to oral environment causing degradation of force by 50%-70% by 1st day. Due to these drawbacks in friction or sliding mechanism, frictionless mechanism is in better position for retraction, as monitoring of optimum force can be done effectively, and it is active for longer duration of time.

FRICTIONLESS MECHANICS

In frictionless mechanism retraction is accompanied with loops or springs which offer more controlled tooth movement than sliding mechanism. In frictionless mechanism when the loops are activated, they distort from their original shape as the tooth moves and then it gradually returns to its undistorted (preactivated) position, delivering the energy stored at the time of activation. It is activated by by pulling the arms of the loop away and cinching them back at the molar tubes. Thus, this approach is friction free, so can be used to move group of teeth more accurately with more precise anchorage control to achieve treatment goals more readily than methods in which friction plays a role.

The Gable bend and Neutral Position

A simple loop when activated is unable to generate adequate counter moment required to achieve the desired tooth movement so preactivation bends also known as gable bends are given which increases the M/F ratio. To maintain the neutral position of the loop which has been altered by the introduction of gable bends, appropriate magnitude and occlusogingival location of the gable bends are vital. Because of the gable bends, the closing loop functions as V- bend in the arch wire. Practically, this means that during canine retraction with the vertical loop placed closed to a canine, a higher ratio M/F ratio would be present on the canine which could better control the apex. The undesirable effect is extrusion of the canine.

Preactivation bends or gable bends can be placed within the archwire or where loop meets the archwire which are placed to increase M/F ratio. For anterior retraction loops should be placed closer to the canine than to the molar and a gable bend should be added near the molar. A gable bend that is larger in the posterior dimension will produce a larger beta moment thus increasing posterior anchorage. For both retraction of anterior and protraction of anterior segment the loop should be placed midway between posterior and anterior segments. A gable bend of equal dimension should be used so that alpha and beta moments are equal and reciprocal space closure occurs. When only posterior protraction is desired, the loop should be located closer to the posterior segment and anterior gable bend should be placed with a greater alpha moment than beta moment, making the anterior teeth the anchorage segment.

METHODS OF CANINE RETRACTION IN
FRICIONLESS MECHANICS

1. Ricketts retraction spring
2. Poul gjessing spring
3. Burstone T loop retraction and attraction spring
4. Marcotte spring
5. Drum spring
6. Opus loop
7. Kalkra simultaneous intrusion retraction spring
8. Wave spring
9. A statistically determined retraction system
10. Niti canine retraction spring

RICKETTS RETRACTION SPRING

Maxillary cuspid retractor is a combination of a double vertical closed helix and an extended crossed T closing loops spring which contains 70mm of wire. (Fig D) It produces 50 grams per mm of activation. The additional wire in its design helps in activation of the spring by contracting the loops and 3 – 4 mm of activation for upper cuspid is sufficient for retraction. Mandibular cuspid spring is a compound spring with a double vertical helical closing loop. It contains 60 mm of wire and produces approximately 75 grams of force per mm of activation. Activation of 2 – 3 mm is required to produce the desired force.

POUL GJESSING SPRING

The Poul Gjessing maxillary canine retraction spring was described by Poul Gjessing in 1985. Essentially the spring consist of a double ovoid helix with a smaller occlusally places helix and is in the preformed version is available commercially constructed in 0.016 x0.022 inch stainless steel wire. The spring is activated by pulling distal to molar tube until the two loops separate (Fig E). The amount of activation produces the recommended initial load of 100 grams. It is critical to avoid over activation of the spring because a few mm of over activation can result in anchor loss. Since the average distance from the centres of the brackets to the CR are identical for the upper and lower canines, the PG retraction spring works equally well for canine retraction in either arch.

BURSTONE T LOOP RETRACTION AND ATTRACTION SPRING

The burstone composite ‘T’ loop retraction spring is made from 0.017 x 0,025-inch TMA wire. T loop retraction spring can be used in group A arches. The attraction springs are used in group B and group C arches. The difference lies in rotational control of the canine, which is achieved with a non-sliding mechanism. Antirotational bends are placed in the retraction assemblies to prevent the canine from rotation as it retracts. It is also possible to use an arch wire to prevent rotation. It is engaged into auxiliary tube of first molar and the vertical tube on the burstone canine bracket. Initially after preactivation controlled tipping occurs (M/F 8:1), as space closes and spring deactivates, the force level decreases so translation occurs (10:1), further deactivation leads to root movements (12:1).

MARCOTTE SPRING

This is a type of minor cuspid retraction spring and is small, light 0.016 closing loop. This spring extends from the auxiliary tube of 1st molar brackets to the bracket on the cuspid and it is activated by being pulled through the auxiliary tube and cinched. The buccal segment feels, then a protractive force and a positive moment, while
the tooth being “walked back” on the wire. Hence are used in group B and group C arches. Activation should be limited to 1-2 mm.

**OPUS LOOP**

This new design can be fabricated from 16 x 22 or 18 x 25 or 17 x 25 TMA wire. The loop is positioned off center about 1.5mm from the mesial canine bracket. It is activated by tightening it distally behind the molar tube and can be adjusted to produce maximum, moderate, or minimum incisor retraction. It also delivers a nonvarying target M/F within the range of 8.0 – 9.1 mm inherently, without adding residual moments by twist or bends anywhere in the archwire or the loop.

**KALRA SIMULTANEOUS INTRUSION RETRACTION SPRING (K – SIR)**

It is made up of continuous 019 x 025 TMA arch wire with closed 7 mm x 2mm U – loops at the extraction site for en masse retraction. The u loop has a 90 degree bend to create equal and opposite moments. A 60 degree V – bend is also located posterior to the center of interbracket distance producing an increased clockwise moment on the first molar.

**A STATICALLY DETERMINATE RETRACTION SYSTEM**

This novel system consisted of a single – force cantilever arm of 017 x 025 TMA for active retraction and a passive rigid stabilizing unit. A turn of helix is placed in front of auxiliary tube for the molar and ended with a hook on its anterior end. A 90 bend is placed in middle of spring. The spring is activated 90 at the helix as well.

**THE WAVE SPRING**

This spring can be used where a closed coil would be appropriate for retraction. It is made up of superplastic nickel titanium alloy delivering large amount of activation about 90g of force from extremely a compact spring only 6mm long in its resting state.

**NITI CANINE RETRACTION SPRING**

The spring is available in 016 x 022 NiTi wire with antitip and antirotational incorporated. It has an ability to deliver continuous force and moments over a broad range on activation.

**RAPID CANINE RETRACTION THROUGH DISTRACTION OF PERIODONTAL LIGAMENT**

Liou and huang in 1998 proposed a concept in which first premolar of the patient is extracted then the interseptal bone distal to canine is undermined by a bone bur. After debridement of that area tooth born custom made distraction device is placed which helps in retraction of canine within three weeks into the extraction space. No complications were also observed during or after the treatment.

**CANINE RETRACTION WITH RARE EARTH MAGNETS**

In a study conducted by John daskalogiannaskisa and Kenneth roy in 1996 they hypothesised that a prolonged constant force provides more effective tooth movement than a impulsive force of a short duration arylene coated neodymium-iron-boron block magnets were used in the experiment. The appliance provided a constant force, and this method was two times faster than the conventional methods of retraction as they use interruptive force which degrade after some time.

**CANINE RETRACTION WITH REMOVABLE APPLIANCES**

Canine retractors are the springs which are used to move the canine in distal direction.

**THE VARIOUS TYPES OF CANINE RETRACTORS ARE AS FOLLOWS:**

Palatal Canine Retractor: The distal movement of the canine teeth can be brought about by a palatal canine retractor if the canine is palatably placed which is made out of 0.6mm stainless steel wire with a coil of 3mm diameter, an active arm and a guide arm. The helix is placed along the long axis of the canine. Activation is done by opening the helix 2mm at a time.

The ‘U’ Loop Canine Retractor: Mechanically it is least effective and used when only minimum retraction of 1-2mm is required. It is made of 0.6mm stainless steel wire. It consists of a U loop, an active arm and a retentive arm which is distal. The base of the U loop is 3mm below the cervical margin. It is activated by closing the U loop. The advantages of this retractor are
its simplicity of fabrication and lesser bulk.

Reverse Loop Buccal or Helical Canine Retractor: It is used when the sulcus is shallow, as in the lower arch. Its flexibility depends on the height of the vertical loop and should be as high as possible. It is made of 0.7mm stainless steel wire. Activation is done by cutting off 1mm of wire from the free end and re-forming it to engage the mesial surface of canine.

Buccal Canine Retractor: The buccal canine retractor is used when the tooth must be moved palatally and distally. It is made of 0.7mm stainless steel wire to provide sufficient strength. It should not be activated by more than 1mm because it is stiff and force decays rapidly as the tooth moves which results in difficulty to maintain continuous tooth movement.

Supported Buccal Retractor: It is made of 0.5mm stainless steel wire supported in a tubing of 0.5mm internal diameter. It is more than twice as flexible as the standard canine retractor, the tubing imparts excellent stability.

RETRACTION IN BEGGS TECHNIQUE
The beggs technique advocates a two-stage retraction. The first stage involves distal tipping of the anterior crowns with elastomers and/or interarch elastics. Begg brackets permit only a point contact between bracket and archwire, so no moment is produces by wire bracket interaction. As a result, uncontrolled tipping of the anterior teeth occurs during the first stage of retraction. The second stage involve lingual torqueing of the anterior roots, usually by means of torquing auxiliary. A moment to force ratio of about 12:1 is required, and such a high ratio is technically difficult to achieve. For this reason, two-stage retraction with initial uncontrolled tipping is not the most efficient retraction method.

Conclusion
Depending upon the condition and severity of malocclusion and treatment techniques employed, a number of methods are used for the retraction of canine either by fixed or removable orthodontic appliances. Every situation requires different technique because of its own limitations. Thus the individual clinician must choose the method he prefers to treat malocclusion which requires tipping or bodily movement or rotation of teeth with minimal time, to produce an aesthetic and functional and near ideal occlusion as much as possible.

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Study of Replantation and Revascularization of Limb and Its Parts

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Abstract

Background: Traumatic amputations may be devastating and can result in irreversible functional and psychological problems in individuals who sustain them. With developmental advances in microvascular surgery, amputated body parts are now routinely attached in many centres across the world. Successful replantation of amputated digits may allow better appearance, better functional outcome and minimal pain to the patient.

Materials and Methods: This is Prospective observational descriptive study comprising of 20 cases with traumatic amputation of extremities and its parts treated with replantation and revascularization of part from October 2014 to February 2017. Nature of injury was classified by Yamano classification of amputation based on mechanism and severity of injury. Patients were evaluated by Chen & Quick DASH score.

Results: The viability of replanted part is guaranteed by a successful vessel anastomosis, while the quality of bone, tendon, nerve and skin repair will determine the overall functional success of replanted parts. Revascularisation procedures had overall better survival than replantation due to less complexity of procedure.

Keywords: Replantation, Revascularization, digit amputation, Microvascular anastomosis.

Introduction

The term replantation is defined as, the reattachment of a body part that has been totally severed from the body without any attachments. Revascularization is the repair or reattachment of a body part which is incompletely amputated from the body and requires vascular (arterial and/or venous) repair. (1)

Traumatic amputations may be devastating and can result in irreversible functional and psychological problems in individuals who sustain them. They occur in all age groups from the children to elderly. So it is imperative that the surgeon be both knowledgeable and meticulous in order to afford the best possible outcome. (2)

When an extremity or part has been severed, it is common desire of both the patient and the surgeon to achieve survival and functional use of replanted extremity.

With developmental advances in microvascular surgery, amputated body parts are now routinely attached.

The ultimate goal of replantation is to restore normal hand or finger function. Successful replantation of amputated digits may allow better appearance, better functional outcome and minimal pain. (3) The surgeon should guide the patient in making the decision by

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explaining the risks involved and likely outcome, secondary procedures and prolonged rehabilitation after the procedure.

**Materials and Methods**

This research is Prospective observational descriptive study conducted in Department of Plastic Surgery, Government Medical College and Sir Sayaji General Hospital, Vadodara. It comprises of 20 cases with traumatic amputation of extremities and its parts treated with replantation and revascularization of part in Department of Plastic Surgery, Medical College Baroda and S.S.G. Hospital, from October 2014 to February 2017.

- Nature of injury was classified by Yamano classification of amputation based on mechanism and severity of injury.\(^{(4)}\)
- Care of amputated part:-
  1) Optimal – Part is wrapped in moist saline gauze and placed in waterproof plastic bag. Plastic bag is placed in ice.
  2) Suboptimal – not following above mentioned standard care of amputated part.

Detailed clinical history and relevant clinical information were recorded and patients have been followed up regularly at 1 week, 1 month and 3 months.

**Inclusion Criteria:**
- Patients with traumatic injuries causing devascularization or amputation of limb and its parts presenting to S.S.G. hospital, vadodara.
  - Patient who are medically fit for surgery

**Exclusion Criteria:**
- Patients with concomitant life threatening injuries.
- Severe crushing or avulsion injury of tissues.
- Multiple segmental injuries in amputated parts.
- Prolonged warm ischemia time (>9hrs).
- Patient who are medically unfit for surgery.
- Mentally unstable patients.

**Surgical Technique:**

Amputated part if not preserved in optimal condition, was wrapped in moist saline gauze and preserved in plastic bag and bag inserted in ice slurry. All partial and near total amputations were assessed for devascularizing injuries. Temporary splintage was given for all associated fractures while patient was transported to operating room. After ruling out all major injuries, if patient is stable for surgery was shifted to operating room.

Feasibility of replantation was judged by examining the amputated part under microscope. Debridement of unhealthy tissue was done and arteries, veins and nerves were identified and tagged with 8-0 nylon. Mid axial incisions were taken on volar and dorsal skin to explore the vessels.

![Figure 1(a) Volar aspect (For vessel exposure)](image)

![Figure 1(b) Dorsal aspect (For vessel exposure)](image)
part and other on extremity simultaneously. Exploration and preparation of extremity was done under operating microscope (TAKAGI OM-10).

**Figure 2- Preparation of amputed extremity**

Depending on amount of soft tissue loss and condition of proximal and distal vessels amount of bone shortening was planned followed by bone fixation, tendon repair, vascular anastomosis and skin closure.

Bone fixation was done with vertical K-wire in all cases of distal amputations while in forearm amputation radius and ulna were fixed with plate and screws. Tendon repair was done with nylon 4-0 with modified Kessler’s sutures and peripheral sutures with nylon 6-0. In zone 2 injuries only flexor digitorum profundus tendon was repaired.

Proximal artery was cut till pulsatile bleeding was seen.

Vascular anastomosis was done with nylon 10-0 round body micropoint needle using 3V S&T clamps for radial and ulnar arteries, 2V S&T clamps for common digital and digital vessels till middle phalanx level and 1V S&T clamps for digital vessels at distal phalanx level. Vessels were anastomosed end to end with simple sutures.(Fig. 3)

**Figure 3- Showing end to end anastomosis between digital vessels**

Heparin injection 1ml (5000IU) bolus followed by heparin drip (5000IU in 500 ml D5 @ 40ml/hr) was started before release of microvascular clamps.

If after proper debridement gap precludes primary repair then interposition vein grafts were used for anastomosis, which were harvested from volar aspect of forearm.(5)

Venous anastomosis was done after arterial anastomosis was found functional and bleeding seen from distal veins. Nerve repair was done with perineural repair technique with nylon 10-0. Skin closure was achieved with primary suturing with or without skin graft. Loose dressings were done with paraffin gauze. Cotton wool dressing was done over the limb. Cast was applied over the dressing, till above elbow maintaining elbow in 90 degree flexion. Limb elevation was given post operatively and adequate hydration was maintained. Heparin drip was continued postoperatively for 5 days. Complete bed rest was given for 5 days.

Smokers and tobacco chewers were advised to stop the habit in post operative period. Patients were monitored and complications such as bleeding, infection, wound dehiscence at surgical site, skin blackening and necrosis (complete or partial), if any, were noted. Post operatively change of dressing was done on day 5 in most of the patients. No case was re-explored. In case of complete necrosis of part, it was managed by shortening closure, debridement and dressings, skin grafting or flap closure.

On follow up, subjective patient evaluation (Quick DASH Score) and functional outcomes (Chen’s criteria for functional outcome) were assessed at 3 months post operatively. Percentage total active motion of survived part was accessed as total active motion in fingers/limb compared to opposite normal finger/limb. Following moments of thumb were noted: flexion and adduction at 1st MCP joint and opposition with little finger, and were described as percentage of movement compared with normal opposite side. Sensory recovery was assessed subjectively and classified in complete, near complete, partial and no recovery, based on patient’s response. (6),(7).
Results

Total 20 patients were taken in study from age 5 to 65 years out of which 4 were females and 16 were males. Commonest mode of injury was machine injury followed by injury by sharp object followed by road traffic accidents. Replantation was done in 9 (43%) patients and revasularization in 12 (75%) patients. One patient had two amputations (middle and ring finger) who underwent revascularization procedure for both amputations.

Out of 12 procedures of revascularization 7 (58.3%) had near total amputation of part of which 1 (14.3%) procedure had complete survival. Among 3 partial amputations 1 (33.3%) had complete survival and 2 (66.7%) had partial survival. Among two patients with deep incised wound with vascular compromise both patients had complete survival of distal part. In replantation procedures one patient had complete survival of distal part and rest were failure.

Mean warm ischemia time in cases of complete survival was 4.8 hours and in cases of failure was 3.9 hours.

Optimal care of amputated part during transportation was taken in only 3 (33%) cases.

In our study 9 (45%) patients were smokers out of which 6 (66.7%) had failure of surgery and 3 (33.3%) had complete survival of distal part. In non-smokers 7 (63.6%) had failure of surgery. Although percentage of failure was high in smokers, it was not statistically significant. (p value of 0.350).

Out of 20 patients 11 (55%) had Yamano type 3 (crush avulsion injury), 7 (35%) had Yamano type 2 (crush cut injury) and 2 (10%) had Yamano type 1 (clean cut injury). Of 13 patients who had failure 9 (69.2%) patients had crush avulsion injury. While only 2 (15.4%) patients of crush avulsion injury had complete survival of part. One patient of clean cut injury had failure.

All patients had single amputation except one who had partial amputation of ring and middle fingers. Amputations were more common on right side of body and on upper limb. 16 (80%) patients had injury on right upper limb of body while only 3 (15%) patients on left upper limb. There was only one case of lower limb amputation at first metatarsophalangeal joint level. Out of 21 total amputations, thumb and little finger were equally affected 5 (23.8%) cases each while only one (4.7%) patient had devascularizing injury over middle finger and one had over palm/dorsum of hand.

Of 21 amputations, most common level of injury was at proximal phalanx with 8 (38%) cases. Out of 8 cases, 1 (12.5%) had complete survival and 2 (25%) had partial survival. 17 (81%) cases were distal to metacarpophalangeal joint level, 1 (4%) case was at metatarsophalangeal joint level and remaining 3 (14.28%) cases had injury proximal to metacarpophalangeal joint. 2 (9.5%) patients had major injuries proximal to wrist joint.

Mean time duration of surgery in failure cases was 7.08 hours while in cases of complete survival was 5.4 hours and 4.5 hours in cases of partial survival.

Thrombosis was most common complication in 16 (76%) patients. Out of 16 patients 9 (56%) had arterial thrombosis and 7 (44%) had venous thrombosis. Of these patients 2 (12.5%) had partial survival of part and rest were failure.

Out of 13 (61.9%) procedures complicated with wound infection 9 (69.2%) had failure, 2 (15.4%) had complete survival and 2 (15.4%) had partial survival. Haemorrhage was seen after 2 (9.5%) procedures. 1 (5%) patient who underwent revascularization of right ring finger had pulp atrophy of the digit.

Salvage procedures—Shortening and closure was most common procedure for defect closure done in 9 (42.8%) procedures. 4 (19%) distant flaps were done (2 groin and 2 abdominal flaps). Groin flaps were done for thumb reconstruction after failed procedure. Debridement and dressing and skin grafting were done in 2 (9.5%) cases each.

Functional recovery (Range of motion):

Functional evaluation of finger motion was done by percentage of total active motion. 4 (80%) patients had percentage TAM more than 70%. One patient (20%) of middle finger revascularization who had partial necrosis of distal part had total active motion of 55%. Patient with successful revascularization at forearm had near
normal (90%) TAM.

Three patients with failed procedure for thumb replantation received reconstruction for thumb. Movements of thumb were measured in four patients of thumb amputation. All patients had adduction more than 70% while three patients had flexion restricted to 50-55% and one patient with thumb tip amputation (Tamai zone 2) had flexion of 95%. All the patients were able to oppose thumb with little finger. Chens grade was measured for functional evaluation in 7 patients with part survival and two patients with thumb reconstruction with groin flap. Of 9 patients 3 (33%) were in grade 1, 5 (56%) patients in grade 3 and 1(11%) in grade 2. No patient had non functional limb (grade 4).

Table 1- Quick DASH (Disabilities of arm, shoulder and hand) score:

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% CI for Mean</th>
<th>Min-</th>
<th>Max-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td></td>
<td>Upper Bound</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minimum</td>
<td></td>
<td>Maximum</td>
</tr>
<tr>
<td>Complete Survival</td>
<td>4</td>
<td>9.65</td>
<td>3.894</td>
<td>1.947</td>
<td>3.45</td>
<td>15.85</td>
<td>4</td>
</tr>
<tr>
<td>Partial Survival</td>
<td>2</td>
<td>26.10</td>
<td>4.808</td>
<td>3.400</td>
<td>-17.10</td>
<td>69.30</td>
<td>23</td>
</tr>
<tr>
<td>Failure</td>
<td>11</td>
<td>18.39</td>
<td>9.670</td>
<td>2.916</td>
<td>11.89</td>
<td>24.89</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>17.24</td>
<td>9.373</td>
<td>2.273</td>
<td>12.42</td>
<td>22.06</td>
<td>4</td>
</tr>
</tbody>
</table>

Although the Mean Quick DASH score was more in partial survival cases, it was not statistically significant.

Table 2 - ANOVA test

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>402.002</td>
<td>2</td>
<td>201.001</td>
<td>2.803</td>
<td>.095</td>
</tr>
<tr>
<td>Within Groups</td>
<td>1003.759</td>
<td>14</td>
<td>71.697</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1405.761</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Age: Youngest patient in our study was 5 years old female with complete amputation at metatarsophalangeal joint of right great toe while eldest patient was 65 years old who sustained near total amputation of right thumb due to assault. Maximum numbers of cases (33%) were present in age group of 21 to 30 years.

In H. Venkatramini et al(9) series similar age distribution of cases was seen with maximum number of cases (33.34%) in age group of 21-30 years.

Sex: In our study there were 16 (80%) male patients and 4 (20%) female patients. In study done by Nadezhda Gavrilova(7) et al 86% cases were males and 14% females. In Mahajan(10) series only 6% cases were females.

It is because males are more outgoing and more prone to machine injury at work and road traffic accidents which are commonest causes of such amputations.

Mode of injury: Injury while working on machine was most common cause of injury in our study which was seen in 6 (30%) patients while road traffic accident
in 4(10%) cases. Assault with sharp object was in only 1 (5%) patient who sustained right thumb amputation.

Two most common injury mechanisms were similar in Njoku Isaac Omoke et al (11) series. Road traffic injuries were higher in their series. There were 30 (56%) road traffic accidents and 7 (13.2%) machine injury cases. Other modes of injury in their series were gunshot, explosives and collapsed building, 3 (5.7%) patients each. The difference may be because their series included all patients of traumatic amputations presented in their hospital, while we included only those cases that met our inclusion criteria for operative procedure.

In study done by Nadezhda Gavrilova et al (3) industrial machinery injury was most common mode of injury seen in 46% cases which is similar to our study.

Lack of awareness and safety measures may be the reason for more cases of machine injuries seen in our study.

**Ischemia time:** All patients presented to us early with mean warm ischemia time was 4.8 hours in 5 complete survival cases while it was 3.46 hours in 13 failure cases. Mean cold ischemia time was 2.3 hours. One patient who presented to hospital after 7 hours of ischemia time had partial survival after revascularization.

**Site of injury:** In our study thumb and index finger were most commonly amputated digits (53.8%), devascularizing injury at the level of metacarpal was seen only in 1 (4.7%) patient and major injuries were seen in 2 (9.5%) patients.

In study done by J.Q.L. Neto et al (12) also, thumb is the most commonly amputated digit. This may be because the thumb is the most important digit for pinch function and rarely the replantation/revascularization of thumb is contraindicated.

**Effect of tobacco and smoking:** In our study of although percentage of failure was high (66.7%) in smokers but result was not statistically significant. There was no statistically significant correlation between tobacco chewing and failure of procedure.

Similar findings were obtained by Ji-Yin He et al. (13) in their study of 149 replants. The study showed that use of cigarettes / tobacco did not affect the replantation outcome.

**Vein graft:** We did not find any significance between use of vein graft and survival of distal part, however study done by Jing Li et al. (14) found statistical significance between use of vein graft and success of procedure.

**Type of amputation and procedure done:** We divided devascularizing injuries of limbs into 4 classes. Complete amputation (part completely separated from limb), near total amputation (part is attached to limb by a small skin tag), partial amputation (part is attached to limb with structures other than skin, with or without skin attachment) and deep incised wound.

Of complete amputations only 1 (11.1%) replantation of little finger at middle phalanx level was successful. Among near total amputations 1 (14.3%) patient with amputation at distal phalanx level was successful. There was no failure case in partial amputation and deep incised wound.

In study by N. Waterhouse (15), success rate was 61% in total amputations and 75% in subtotal amputations.

In our study 85% patients had severe crush injury and 55% patients had avulsion component. Replantation and revascularization was attempted in all cases presented and criteria for selection was not rigid.

81% cases in our study were distal to metacarpophalangeal joint involving digital arteries which are technically more demanding.

In 67% cases care of amputated part was suboptimal.

Mean time duration of surgery in failure cases was 7.08 hours which increased intraoperative ischemia time.

Replantation and revascularization procedures are technically demanding and there is longer learning curve.

**Complications:** In our study thrombosis occurred in 16 digits of which 9 (56%) had arterial thrombosis and 7 (44%) had venous thrombosis. Wound infection was seen in 13 (62%) patients. Two patients had reactionary haemorrhage, probably because of slippage of ligature, these patients were managed conservatively. One patient developed pulp atrophy in follow up. Skin grafting was
done as salvage procedure in 2 (9.5%) patients. Distant flaps (groin and abdominal) were done in 4 (19%) failed procedures, for thumb reconstruction. Shortening and closure was done in 9 (42.8%) procedures.

In N Waterhouse (15) series thrombosis was seen in 13 digits (31%) of which 11 (85%) had arterial thrombosis and 2 (15%) had venous thrombosis. local flaps (cross finger flap) were done in 2 (4.7%) digits. 2 (4.7%) digits had infection along track of k-wire. All patients had some degree of atrophy in fingers.

In N Waterhouse study, all cases of thrombosis were explored and they could salvage 2 cases from 13 cases of thrombosis. In our study we did not re-explore any case of thrombosis due to lack of patient willingness and motivation and it was difficult to manage re-exploration with limited human resources. (15)

Raja Sabapathy S et. al. (16) in their study of ring avulsion amputations found raw areas at wound margins in 6 (75%) patients who required split skin grafting. One patient had partial necrosis of volar skin managed by cross finger flap.

**Total Active motion:**

In 4 (80%) survived fingers, range of motion was >70% while only 1 (20%) patient had range of motion in 50-70%.

In Mahajan et al (10) study range of motion was >70% for 2(14.28%) cases and 50% in 4(28.67%) cases.

The reason for difference being we studied all devascularizing injuries i.e. both replantation and revascularization and in Mahajan et al study only replanted hand at wrist were studied.

We studied thumb function as flexion, adduction and opposition. All patients in our study had adduction >70%. One patient in our study having thumb tip amputation had flexion >70%. All patients were able to oppose thumb with little finger. In Mahajan et al (10) study opposition was >70% in 3 (21.42%) patients. Reason for difference may be because of preservation of 1st metacarpophalangeal joint in all patients of our study.

**Chen classification: (Table 3 - Comparision with other studies)**

<table>
<thead>
<tr>
<th>Chen grade</th>
<th>Present series</th>
<th>B. Yaffe et al(17)</th>
<th>Sabapathy S.R et al(15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>3 (33%)</td>
<td>2 (33.33%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1 (11%)</td>
<td>2 (33.33%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>5 (56%)</td>
<td>1 (16.67%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0 (0%)</td>
<td>1 (16.67%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

In our study we included both major as well as minor amputations. Chen grade (8) was measured for both. No patient in our study had cold intolerance. Out of 9 patients three were treated by distant flaps, two with groin flaps for thumb reconstruction and 1 with abdominal flap for thumb tip reconstruction. Although %TAM and power was more but there were no sensations in the flap area and so were grouped in grade 3. Three (33%) patients had grade 1 function (excellent outcome), while no patient had non functional limb, grade 4 (poor outcome).

B. Yaffe et al series and Sabapathy SR series considered only major upper limb replant.

In B Yaffe et al series 2(33.3%) patients had excellent outcome and 1(16.67%) had poor outcome.

In Sabapathy SR series majority, 9(45%) patients had grade 2 (good outcome), while 2(10%) had non functional limb.

**Quick DASH:**
Quick DASH compares the postoperative result with the patient’s functional competence in day to day activities.

Quick DASH score was calculated in 17 patients out of 20 as one patient was lost to follow up, in one patient 3 months follow up is still awaited and one patient had amputation of great toe.

In our study mean quick DASH score in cases of complete survival was 9.65, for failure cases it was 18.39, and in partial survival cases it was 26.1.

In study done by J. Dabernig et al (18) after successful replantation DASH Score achieved was 12.3. while in Hass F et al (19) Quick DASH score was 11.3. Findings were similar to our results. Quick DASH score of partial survival cases were high because it took longer time for wound to heal following partial necrosis of skin which delayed physiotherapy.

**Conclusion:**

Traumatic amputations were more common in young, working and economically productive age group, so successful replantation and revascularization procedures helps to reduce the financial burden on them.

First responders should be adequately educated regarding optimal care of amputated parts.

There are multiple factors to be considered for replantation and revascularization procedures. Preoperative selection includes ischemia time, contamination and care for preservation of part, avulsion and crushing injuries. Intraoperative management includes number of arteries, veins and nerve repaired, use of vein graft, bone shortening, anticoagulation and postoperative rehabilitation including physiotherapy, which independently influence the overall outcome of replantation and revascularization cases.

The viability of replanted part is guaranteed by a successful vessel anastomosis, while the quality of bone, tendon, nerve and skin repair will determine the overall functional success of replanted parts.

For thumb amputations, replantation and revascularisation is best option for thumb reconstruction and should be attempted in all cases.

Revascularisation procedures had overall better survival than replantation due to less complexity of procedure.

These procedures require specialized microsurgical skills and appropriate use of microvascular techniques, ultra-modern microscope, pneumatic drills and fine instruments for providing better results.

Well-developed replantation centre and multiple skilled replantation teams is need of modern time for assurance of good success rate.

**Conflict of Interest:** None

**Funding:** None

**Ethical Approval:** Ethical clearance for study was taken from ethical committee of Baroda Medical College, Vadodara before conducting this study.

**Acknowledgments:** We acknowledge the support and cooperation of all the residents and head of department of plastic surgery at Baroda Medical College and all patients included in this study.

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Reliability of Different RBC Indices and Formulas in the Discrimination of β-Thalassemia Minor and Iron Deficiency Anemia in Surabaya, Indonesia

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Abstract

In this study, we evaluated the RBC indices in differentiating β-thalassemia minor and iron deficiency anemia in a healthcare center. This was an observational analytic study with a cross-sectional design using blood specimens of children aged 3 years to 17 years with microcytic hypochromic anemia based on the results of complete blood counts and evaluation of peripheral blood smears. Calculation of the RBC indices was performed as an initial screening to differentiate β-thalassemia minor and iron deficiency anemia. Iron profile examination and hemoglobin electrophoresis were performed to confirm the diagnosis of β-thalassemia minor and iron deficiency anemia. The results of the independent samples t-test showed significant differences in Hb, MCV, MCH, and MCHC between β-thalassemia minor and iron deficiency anemia (p <0.05). The results of the Mann Whitney test showed a significant difference in the Mentzer Index to distinguish between β-thalassemia minor and iron deficiency anemia (p <0.05). Also, the results of the independent samples t-test showed significant differences in the Green & King formula, Sirdah et al. formula, and the Maltos and Carvalho Index (p <0.05). The Green and King formula has a diagnostic sensitivity of 78.6% and a specificity of 76.6% and an accuracy of 78.03%. Various formulas based on the results of complete blood count parameters have been developed to detect β-thalassemia minor in areas with a high prevalence of β-thalassemia with different sensitivity and specificity. In this study, it showed that the Green and King formula has a diagnostic sensitivity of 78.6% and a specificity of 76.6%, and an accuracy of 78.03%. Green and King’s formula can be applied as an initial screening to differentiate β-thalassemia minor and iron deficiency anemia.

Keywords: RBC indices, β-thalassemia minor, iron deficiency anemia.

Introduction

WHO study shows that as many as 800 million children and women have anemia, which the population with the highest prevalence of anemia and the lowest hemoglobin concentration being in the Southeast Asian, Eastern Mediterranean, and African regions. Approximately 50% of cases of anemia are considered to be due to iron deficiency, other causes are inherited or acquired disorders that affect hemoglobin synthesis, red blood cell production, or red blood cell survival.[¹]

The results of Basic Health Research from the Ministry of Health of the Republic of Indonesia (2013) have shown that 21.7% of the age of population ≥1 year, 28.1% of toddlers aged 12-59 months, and 37.1% of pregnant women are the proportion of anemia in Indonesia.[²] Differential diagnosis of microcytic hypochromic anemia is very important to be considered because the interpretation of its peripheral blood smear...
can be found in iron deficiency anemia and β-thalassemia trait.[3,4] Iron deficiency and β-thalassemia minor are best differentiated using serum ferritin level, serum iron level, total iron-binding capacity, transferrin saturation, and Hb A₂ level, along with a complete blood count (CBC) and examination of a peripheral blood film.[3] Carriers of β-thalassemia are usually clinically asymptomatic. However, they have characteristics of the CBCs with mean corpuscular volume (MCV) less than 80 fL and mean corpuscular hemoglobin (MCH) less than 27 pg.[5]

The ability and experience of a doctor in discriminating between β-thalassemia minor and iron deficiency anemia (IDA) clinically is very important because there are still difficulties in distinguishing between these two diseases. There are differences in the prognosis and management of those diseases, so the exclusion of iron deficiency anemia could be achieved mathematically using the red blood cell (RBC) parameters.[4,5] Various formulas using a complete blood count have been developed to detect β-thalassemia carriers in areas where the prevalence of thalassemia major is high; however, specificity and sensitivity vary across regions.[4,6]

In an attempt to simplify the differential diagnosis between IDA and β-thalassemia minor, several indices using blood cell count parameters have been suggested.[4] According to many studies, the formula developed by Green and King \[\frac{\left((MCV^2 \times RDW)\right)}{Hb \times 100}\], Sirdah et al. Formula \[(MCV – RBC – 3 \times Hb)\], Matos and Carvalho Index \[(1.91 \times RBC) + (0.44 \times MCHC)\], and Mentzer Index \[MCV/RBC\] have a good performance.[4,5,7] Capillary zone electrophoresis (CE) was able to detect the most common variants but differed concerning measurement HbA. The CE was able to measure HbA₂ in the presence of HbE. This is important to diagnose thalassemia and hemoglobinopathies.[8] Few data are available about the evaluation of the diagnostic reliability of different RBC indices and formulas to differentiate of the β-thalassemia minor and iron deficiency anemia (IDA) in Surabaya, Indonesia. In population or mass-screening programs especially in developing countries, these hematology indices could be applied where resources are limited.[7] Therefore, this study aimed to evaluate the sensitivity and specificity of different RBC indices and formulas in the differentiation of the β-thalassemia minor and iron deficiency anemia.

### Materials and Methods

#### Study Subjects and Sample Collection

This was an observational study with a cross-sectional approach performed in all blood specimens in patients aged 3 years until 17 years with hypochromic (MCH <27 pg) microcytic (MCV <80 µl) anemia based on the results of a complete blood count (CBC) and evaluation of peripheral blood smears. A total of 223 subjects (124 males, 99 females) were studied in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia between January 2019 and August 2019. The hematological index calculation (Mentzer Index, Green and King Formula, Sirdah et al. Formula, and Matos and Carvalho Index) was performed as initial screening in distinguishing iron deficiency anemia and β-thalassemia minor. Examination of iron profiles (serum iron, total iron-binding capacity, and serum ferritin), and hemoglobin electrophoresis was carried out subsequently as confirmation of the diagnosis of iron deficiency anemia and β-thalassemia minor.

Inclusion criteria for iron deficiency anemia (IDA) subjects were microcytic hypochromic anemia with serum iron level <50 µg/dl, TIBC level >390 µg/dl, transferrin saturation <15%, and serum ferritin level <15 µg/L.[9] In addition, inclusion criteria for the β-thalassemia minor (BTMi) subjects were children with microcytic hypochromic anemia, had a family history (parents or siblings) of β-thalassemia. Their CBCs show hemoglobin (Hb) less than 13.5 g/dl, MCV less than 80 µl, and MCH less than 27 pg. Subjects with severe sepsis were not included in this study and none of the subjects had a combined case of β-thalassemia minor and iron deficiency anemia. Then, β-thalassemia minor is confirmed by quantitation of HbA₂, in which HbA₂ >3.5% indicates β-thalassemia minor.[3] The following indices and formulas were calculated and compared:

Green and King formula \[(\frac{\left((MCV^2 \times RDW)\right)}{Hb \times 100})\].[10]  
Sirdah et al. Formula \[(MCV – RBC – 3 \times Hb)\].[7]  
Matos and Carvalho Index \[(1.91 \times RBC) + (0.44 \times MCHC)\].[4]  
Mentzer Index \[MCV/RBC\].[11]
Sensitivity [true positives/(true positives + false negatives)], specificity [true negatives/(true negatives + false positives)], positive predictive value [true positives/(true positives + false positives)], negative predictive value [true negatives/(true negatives + false negatives)], and accuracy [(true positives + true negatives)/total subjects] were calculated for each index and formula at wide-range of cut-off values.

Sample Preparation, Hematological and Clinical Chemistry Evaluations

Peripheral blood (3 mL) was collected in tubes containing K3EDTA (ethylenediaminetetraacetic acid) with a final concentration of 1.5 mg/mL. Complete blood count was examined using an automated hematology analyzer (CELL-DYN Ruby, Abbott® and Sysmex® XN-1000) in the laboratory unit of clinical pathology, Dr. Soetomo General Academic Hospital.

Hemoglobin electrophoresis tests were performed using a capillary electrophoresis system (MINICAP Sebia®) which able to measure HbA₂ in the presence of HbE. The capillary electrophoresis system does not require daily calibration, but normal HbA and HbA₂ migration controls are analyzed through each capillary daily before running QC materials or patient samples to ensure proper charge and function of the capillaries.[12] Normal HbA₂ control (Sebia®, France) for QC materials were also run daily.

Another sample of the peripheral blood (2 mL) was collected with BD Vacutainer® SST™ blood collection tubes contain spray-coated silica to aid in clotting and a polymer gel for serum separation to evaluate serum iron, total iron-binding capacity, and serum ferritin values.

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for each measurement of the formulas. Descriptive statistics such as the mean, the median, and the standard deviation (SD) were calculated for hematological parameters (RBC, hematocrit, RDW-CV) and also age variable. The differences between the two groups parameter (β-thalassemia minor and iron deficiency anemia) were compared using the Mann-Whitney U test, because of the distribution of these parameters were non-normal. The independent samples t-test was used to compare the differences for hematological parameters (Hb, MCV, MCH, and MCHC) between these groups. The normality of data was evaluated using the Kolmogorov-Smirnov test.

Results

The Hb value in the BTMi group was 8.53±1.62 compared to the IDA group was 10.96±2.13 (p <0.001). MCV values of 71.95±6.76 and MCHC 30.96±1.86 in the BTMi group were lower than in the IDA group (76.48±4.85 and 32.20±1.45 with p <0.001). RDW-CV in the BTMi group was 20.15±4.77, significantly higher than the IDA group (14.6±3.28 with p <0.001). Erythrocyte count (RBC) was higher in the IDA group of 4.46±8.86 than in the BTMi group (3.86±0.81 with p <0.001) (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BTMi (n: 159)</th>
<th>IDA (n: 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>4.49-14</td>
<td>8.53±1.62*</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>13.6-43.8*</td>
<td>27.57±5.06</td>
</tr>
<tr>
<td>RBC (x10⁶/µL)</td>
<td>1.9-6.77*</td>
<td>3.86±0.81</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>55.0-99.3</td>
<td>71.95±6.76*</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.6-31.7</td>
<td>22.34±3.02*</td>
</tr>
<tr>
<td>MCHC</td>
<td>26.5-34.9</td>
<td>30.96±1.86*</td>
</tr>
<tr>
<td>RDW-CV (%)</td>
<td>8.3-34*</td>
<td>20.15±4.77</td>
</tr>
</tbody>
</table>
Note: Hb: hemoglobin; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-CV: red cell distribution width-coefficient of variation.

*Significant, \( p < 0.001 \)

Table 2. Evaluation of different RBC indices in for distinguishing in \( \beta \)-thalassemia minor and iron deficiency anemia.

<table>
<thead>
<tr>
<th>RBC Indices or Formula</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
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</thead>
<tbody>
<tr>
<td>Mentzer Index</td>
<td>66%</td>
<td>62.5%</td>
<td>81.4%</td>
<td>42.6%</td>
<td>65.02%</td>
</tr>
<tr>
<td>Green and King Formula</td>
<td>78.6%</td>
<td>76.7%</td>
<td>89.3%</td>
<td>59%</td>
<td>78.03%</td>
</tr>
<tr>
<td>Sirdah et al. Formula</td>
<td>64.2%</td>
<td>64.1%</td>
<td>81.6%</td>
<td>41.8%</td>
<td>64.13%</td>
</tr>
<tr>
<td>Martos and Carvalho Index</td>
<td>27.7%</td>
<td>25%</td>
<td>47.8%</td>
<td>12.2%</td>
<td>26.9%</td>
</tr>
</tbody>
</table>

The data from the present study (Table 2) showed the Green and King formula has the highest sensitivity (78.6%) and specificity (76.6%) in the differentiation of BTMi and IDA. The highest PPV value was found in the Green and King formula (89.3%) and the lowest was in the Martos and Carvalho index (47.8%). The highest NPV value was found in the Green and King formula (59%) and the lowest was in the Martos and Carvalho index (12.2%). There were none of the differentiation indices with high sensitivity and specificity (100%) in differentiating BTMi and IDA, but the overall index was significant in differentiating BTMi and IDA (\( p < 0.05 \)) against the gold standard examination of hemoglobin electrophoresis (HbA2).

Discussion

The ability and experience of a doctor in distinguishing between BTMi and IDA is very important because it has clinical implications where the two diseases have different aspects of etiology, prognosis, and management.\[^{13}\] The most common causes of anemia are iron deficiency anemia (IDA) and thalassemia trait (TT).\[^{1}\] In infants, the incidence of IDA is estimated to be between 20-25%. The results of the Basic Health Research of the Ministry of Health of the Republic of Indonesia (2013) showed that the proportion of anemia in toddlers aged 12-59 months is 28.1%.\[^{2,14}\] Many investigators still have used hematological index calculations based on complete blood parameters as the initial screening to differentiate BTMi and IDA.

A capillary electrophoresis system for the examination of hemoglobin electrophoresis costs more than a routine CBC, and if we can routinely include an automatic alert signal in any routine CBC analyzed by the hematology analyzer, the referring physicians can get an indication of whether they need to perform further studies.\[^{15}\]

The data from the present study (Table 1) showed significant differences in hematologic parameters between the BTMi and IDA groups. The higher RBC increase in the IDA group compared to the BTMi group was probably related to the administration of iron therapy in children with IDA. Vehapoglu et al. (2014) observed that the RBC increased in patients with IDA at the time of initiation of iron therapy and a decreased of the RBC at the end of iron therapy.\[^{13}\] This is consistent with the study of Aslan and Altay (2003) that 36 out of 140 (26%) children aged 6 months to 48 months with nutritional IDA without bleeding, experienced a
significant increase in RBC accompanied by an increase in Hb and Het, as well as a decrease in MCV values. A continuous increased in RBC occurred in patients with higher RBC (high-RBC patients) up to 4 weeks of iron therapy compared with those with lower RBC (low-RBC patients).[16]

Evaluation of the hematological indices in this study showed the Green and King formula had the highest sensitivity (78.6%) and specificity (76.6%) in differentiating BTMi and IDA, followed by the Mentzer index (66% and 62.5%), the Sirdah et al. formula (64.2% and 64.1%), and the Martos and Carvalho index (27.7% and 25%). These results were in accordance with the study of Urrechaga et al. (2011) who showed that the Green and King formula had high sensitivity (91%), specificity (99.1%), Youden index (90.1%), and area under the curve (AUC) in differentiating BTMi and IDA.[17]

This study had several limitations such as no mutational analysis data was carried out in the study. The platelet parameters were not included in this study which are available in the CBCs result that may be helpful as discriminating guide for BTMi and IDA.

**Conclusion**

In summary, it was concluded that none of the differentiation indices or formulas provided 100% sensitivity and specificity for the discrimination of BTMi and IDA. For the population of children in Surabaya, Indonesia, applying the Green and King formula might be useful in the early or initial screening stages of children with the hypochromic microcytic anemia.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Source of Funding:** This study was supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia.

**Acknowledgements:** We thank Arif Nur Muhammad Ansori for editing the manuscript.

**Ethical Approval:** This study was approved by the Health Research Ethics Committees (KEPK) of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ethical Clearance Number: 1147/KEPK/IV/2019).

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The Effect of Interleukin-10 and Its Relationship with The Level of White Blood Cells in Women Spontaneous Miscarriage Undergoing Intracytoplasmic Sperm Injection (ICSI) Technique

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Abstract

This study was conducted on 84 samples of women subject to the ICSI program. Blood sample was taken to measure WBC count, Interleukin-10 and β-hCG levels. Groups were divided based on the β-hCG level to two groups (Pregnant Women Group - Pregnancy Failure Group) and the total of pregnant women divided into (Pregnancy Group and spontaneous miscarriage).

The results of the current research showed a significant increase in the level β-hCG and that the increase in the level of this hormone is evidence of the presence of high success rates for pregnancy in women who performed operations IVF, where the success rate at the beginning of the matter reached 61.9%, after which it decreased to 33.3% after the first three months due to the occurrence of spontaneous miscarriage of pregnant women due to various immunological and physiological reasons, as well as a positive correlation between the level of β-hCG and other parameters within the study (Interleukin-10 -WBC).

The results of the current research also showed a significant difference between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continued pregnancy) in relation to the level of Interleukin-10, Also, The results of the current research showed a significant difference between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continuation of pregnancy) in relation to the level of WBC, and the present study found a positive relationship between the level of Interleukin-10 and WBC.

Keywords: miscarriage, ICSI technique, Interleukin-10, white blood cells.

Introduction

Infertility is a widespread disease worldwide and it means “the inability of the spouses to achieve pregnancy within one year of marriage” and the estimated rate of infertility in the world is around 15-20% (¹).

In vitro fertilization, intracytoplasmic sperm injection (ICSI), and intrauterine insemination (IUI) be are the main methods of assisted reproductive technology (ART). Found several Various studies in recent years have indicated that occur risk factor for implantation failure of women after In vitro fertilization - ICSI which may be immunological parameters or biochemical parameters may be affected on In vitro fertilization intracytoplasmic sperm injection results (¹⁵).

White blood cells (WBCs) They are considered an important part of the immune system in the body. It should be noted that the increase in the number of white
blood cells or their lack of a normal limit indicates the presence of a health problem that afflicts the patient, hence cause of white blood cells rise for a number of reasons, the most important of which are summarized below. For pregnancy and childbirth or spontaneous miscarriage because increase Infection in Urinary tract with or immune system problem (2,16).

Interleukin -10 has several effects and has many roles in regulating the body’s immunity as well as affecting inflammation and is involved in stimulating phagocytic cells as well as has a role in the survival of B cells and building antibodies. There are many studies that have demonstrated that interleukin-10 inhibits fatty polysaccharide and is also stimulated by the bacterial product of pro-inflammatory (3).

Materials and Methods

This study was conducted in the laboratories of the Department of Biology, College of Science, University of Kufa, and in the Laboratory of the Fertility Center in Sadr City Medical City in Najaf Governorate / Najaf Health Directorate / Ministry of Health / Iraq.

Taked about five milliliters of intravenous blood samples were drawn in the morning from women which undergoing intracytoplasmic sperm injection (ICSI) technique during three parts, the first part after 14 day from injection, the second part after trimester spontaneous miscarriage while the third part for women which be continuous pregnant and using a needle and syringes which is used for one time from each patient and control. Four milliliters of the blood then it was left in a gel tube at room temperature for 10 minutes to complete the blood clotting, then centrifuged at 3000 rpm for 5 minutes, then the serum was separated for measurements of β-hCG level and Interleukin-10 level by ELISA method and one milliliter of the blood was put in EDTA-Na2 treated collection tubes for measurements of number of white blood cells count by Genex Hematology Analyzer.

Statistical Analysis

The popular statistical system (Graph Pad prism ver. 5) was adopted, and a one-way analysis of variance - Anova method (by Tukey’s multi-comparative test) was used to compare the groups divided into the measured parameters. The results are expressed as (Mean ± Standard Error). Correlation coefficients were calculated to estimate the correlation between tags and parameters. Descriptive statistics and correlation coefficients were performed using mega stat (V10.12 version) for excel 2010 (4).

Results

In this test showed found significant difference (p<0.05) between pregnant women which was 52 (4.24±0.25) and non-pregnant women (Implantation Failure) (2.56 ±0.10) which was 32 from women which undergoing intracytoplasmic sperm injection technique as shown in table (1).

| Table (1): Result β- Human chorionic gonadotropin hormone test which differ between pregnant women which was 52 and non- pregnant women (Implantation Failure) which was 32 from women which undergoing intracytoplasmic sperm injection technique |
|---|---|---|---|
| Groups | Total Number | hCG (ml U/ml) Mean ± SEM | P value |
| Negative (Implantation Failure) | 32 | 2.56 ± 0.1 | 0.001 |
| Positive (Pregnant) | 52 | 2.24 ± 0.25 | |

In this test showed found significant difference (p<0.05) between Continuous pregnant women (control group) which was 28 women and non-pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure) (32) and found non significant difference with spontaneous miscarriage (24) as shown in table (2) .
Table (2): Results of White blood cell Count test which differ between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and spontaneous miscarriage (24)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Number</th>
<th>WBC Count (cell/ml) Mean ± SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy Failure</td>
<td>32</td>
<td>9442 ± 473.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Spontaneous Miscarriage</td>
<td>24</td>
<td>13860 ± 428.1</td>
<td></td>
</tr>
<tr>
<td>Continued Pregnancy</td>
<td>28</td>
<td>7765 ± 331.8</td>
<td></td>
</tr>
</tbody>
</table>

In this test showed found significant difference (p<0.05) between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and spontaneous miscarriage (24) ) as shown in table (3).

Table (3) : Results of Interleukin-10 test which differ between Continuous pregnant women (control group) which was 28 women and non- pregnant women groups which was 56 from women which undergoing intracytoplasmic sperm injection technique (Implantation Failure (32) and spontaneous miscarriage (24) ).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Number</th>
<th>IL-10 (pg/ml) Mean ± SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy Failure</td>
<td>32</td>
<td>11.99 ± 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Spontaneous Miscarriage</td>
<td>24</td>
<td>13.83 ± 0.75</td>
<td></td>
</tr>
<tr>
<td>Continued Pregnancy</td>
<td>28</td>
<td>6.16 ± 0.72</td>
<td></td>
</tr>
</tbody>
</table>

The study showed the presence of a positive correlation between, Interleukin-10 with White blood cells count as in table (4).

Table (4): The correlation between Interleukin-10 with WBC Count.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-10</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC Count</td>
<td>r = 0.745</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Discussion

The results of the current research showed a significant increase in the level of significance (p<0.05) in the level of the β- Human chorionic gonadotropin hormone, and the reason for this is that this hormone is evidence of the presence of pregnancy in women who underwent ICSI operations may be because it is excreted mainly from the placenta during the formation of the fetus and this study is consistent with what reached by some authors in this regard and that the increase in the level of this hormone is evidence of high success rates of pregnancy for women who conducted ICSI operations, where the success rate initially reached 61.9%, after which it decreased to 33.3% after the first three months due to the occurrence of spontaneous abortions for women. Pregnant women for various immunological and physiological reasons, as this study indicated, and these results are consistent with what was reached.
The results of the current research showed a significant increase at the level of significance (p<0.05) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continued pregnancy) in the concentration of interleukin-10 in When the differences were not significant between the group (pregnancy failure) and the group (spontaneous miscarriage may be because the pregnant women have a low white blood cells level compared to aborted women because white blood cells is evidence of infections in the genital and urinary tract as a result of the spontaneous miscarriage process and Interleukin-10 is one of the cytokines that stimulates the occurrence of infections and is responsible for the start of the inflammatory process and is called the inflammatory station The fourth of the cells devoured and through the diagnosis of the concentration of interleukins, the reaction of the immune system can be known as normal, high or low, and it is consistent with its findings (9,10).

As it was explained by increasing the level of interleukin-10 in aborted women, it is an indication of the role of interleukins whose function is the communication between immune cells, whose action is directed at defending the body.

The results of the current research showed a significant increase at the level of significance (p<0.05) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continued pregnancy) with respect to white blood cells count while The differences were significant between the (pregnancy failure) group and the (spontaneous miscarriage) group, and this study is consistent with the findings of (11,12) and this result may be explained that the reason for this is that pregnant women have a low white blood cells count level compared to aborted women This may be because white blood cells count is evidence of the presence of infections in the genital and urinary tract as a result of the abortion process, where these results are consistent with what was reached (11,14) where he explained the presence of a significant increase in white blood cells women abortifacients and pregnancy after a period when the failure of vaccination compared to non-spontaneous miscarriage women.

The current study also showed a positive correlation between the level of interleukin-10 and WBC and perhaps the reason for this is that the increase in the level of white blood cells during pregnancy or spontaneous miscarriage in women, which is often accompanied by an increase in urinary and genital tract infections which leads to an increase in the level of interleukin - 10 (15,16).

Conclusions

The level of interleukin - 10, and increase in the level of significance (p<0.05) in the level of the β- Human chorionic gonadotropin hormone and The results of the current research showed a significant increase at the level of significance (p<0.05) between the group (pregnancy failure) and the group (spontaneous miscarriage) compared with the control group (continued pregnancy) with respect to white blood cells count.

Acknowledgements

This work was achieved at Kufa university, faculty of science, biology department, and al-Sajjad general hospital, in addition to workers and all participants. We would like to express our heartfelt thanks to those who helped us through this study.

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Constitutional Right to Vote for People with Mental Disorders (PMWD) with Case Study in Yogyakarta, Indonesia

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Abstract
The right to vote as one of the constitutional rights is certainly in favor of all Indonesian citizens, including those who suffer mental disorders. The Constitutional Court through the Constitutional Court Verdict Number 135/PUU-XIII/2015 has stated people with mental disorders, as long as their condition is not permanent, they can vote. However, this verdict has the potential to violate the constitutional rights of the citizen. This research location will be carried out in the Special Region of Yogyakarta where it is one of the provinces with the largest mental disorder communities in Indonesia. This research uses a normative juridical method and focusing on literature studies as its main sources. This research will conclude about the method used by people with mental disorders to access their right to vote. Furthermore, it will also explain the extent to which the Constitutional Court’s decision has an impact on patients with psychiatric disorders in the Special Province of Yogyakarta.

Keyword: constitutional rights; right to vote; people with mental disorder

Introduction
Political rights contain the nature of universality and equality, which means that everyone has the same opportunity in the electoral process, arranges political access that is not limited by anything, and channels references that are owned by each person through voting mechanism. This is in line with what has been mandated in Law Number 18 of 2014 concerning Mental Health, which in Article 2 letter h states that one of the principles implemented in the Law is the principle of non-discrimination. Article 3 of the same law also guarantees the rights of a citizen suffering from mental disorders. This article then aims to find out how persons with disabilities, especially persons with disabilities with the category of mental retardation, channel their right to vote through general elections. Especially considering that political rights (to vote and be elected) are under gable rights for everyone and without discrimination.

Thus, it can be seen that every Indonesian citizen has the right to vote regardless of the condition of each person. In general, Indonesia’s General Election Commission (KPU) has established special regulations that provide opportunities for persons with disabilities to be able to exercise their rights. The KPU has basically provided a means for persons with this type of mental retardation to be able to channel their constitutional rights. This is then an elaboration of the Constitutional Court Decision Number 135/PUU-XIII/2015 which in its decision states that as long as a person does not have a mental illness that can permanently affect a person’s choice, then that person still has his constitutional right to vote. The problem here is that the Constitutional Court decision has the potential to injure the constitutional rights of citizens, especially someone suffering from mental disorders. The Court’s decision has great potential to injure the constitutional rights of citizens, especially someone suffering from mental disorders. The Court’s decision has great potential to injure the constitutional rights of a person with a mental health disorder, especially their right to vote. It is possible for people with mental disorders to recover, although it requires strict assistance and a long process. Yogyakarta Province based on Basic Health Research (Riskesdas) is choose as research location as it has the number of people with psychiatric disorders that
has increased from a number of 2.8% in 2013 increased quite high to 10% in 2018. This makes Yogyakarta Province occupy the 2nd position with the largest number of people suffering from psychiatric disorders. This explains that there are 10 people with psychiatric disorders every 1000 people in Yogyakarta Province. This illustrates the legal conditions that occur in the field that there are a number of people in Yogyakarta Province who have the potential to be impaired by their right to vote, which is a citizen’s constitutional right as a result of the Constitutional Court Decision.

Research Methods

This research is a conceptual research with normative juridical research. Normative legal research is legal research that puts down the principles, values and legal norms that are contained in statutory regulations, as well as court decisions. The approach used in this paper is a statutory approach. The technique of collecting legal materials in this study was carried out by using literature study techniques. This research uses the deductive legal material analysis method, in which the legal materials that have been collected are classified and then conclusions are drawn from general matters into specific matters.

Urgency of Inclusive Elections for Patients with Psychiatric Disorders

The implementation of inclusive elections is very relevant to be implemented in Indonesia, considering that the right to vote is not only owned by normal people, but also for those with disabilities. The inclusiveness of an election can be said to be successful when everyone who has the right to vote can participate in the electoral process by selecting the party who is most deemed capable of solving their problem. One indicator that an election can be said to be of quality is the large level of community participation from all groups in an overall electoral process. Including those with psychiatric disorders, the implementation of inclusive elections in Indonesia itself is far from being feasible. The “human side” of state administrators has not yet grown to provide all the needs of voters with this special category, making it very difficult for them to be able to exercise their rights.

Realizing inclusiveness in the general election process itself is not as easy as turning your hand. Reflecting on the electoral process that was carried out by the Bolivian state in 2002, the participation of both parties is very strong to be able to realize inclusiveness in general elections. Apart from the participation of the parties, inclusiveness also needs to be achieved in several ways, including: fundamental changes in the electoral system, understanding of the ideal conception of elections (through an appropriate electoral education process), and social consolidation of those who wish to be included in the concept. Inclusiveness of elections that are being formed. The failure of the process of inclusiveness can be the beginning of a disaster that general elections, where elections are only held as a formal process to maintain power from a group of people who easily gain power.

Participation of People with Mental Disorders in Yogyakarta in the General Election

According to research conducted by the Provincial Health Office of Yogyakarta in 1999, of the 3.5 million residents of the Province of Yogyakarta, 12,322 of them were sufferers of psychiatric disorders. The results of the 2017 Yogyakarta regional head election show that the turnout rate for voters with disabilities has increased compared to the results of the last election in 2014. The results of the 2017 Yogyakarta regional elections were 350 out of it. 494 persons with disabilities registered in the Permanent Voters List (DPT) who exercised their voting rights. This means that the percentage of persons with disabilities recorded in the DPT who exercised their voting rights in the 2017 Yogyakarta city elections reached 70.9%.

Seeing the position of the participation of persons with mental disorders in the general election can be examined from 2 points of view, philosophical and sociological. Philosophically, the revision of Law Number 8 of 2015 refers to the consideration that all basic human rights must be fulfilled, without exception. Despite experiencing conditions of limited mental illness, every Indonesian citizen has rights directly guaranteed by the Constitution. Sociologically, the role of the state is very important to create political justice for every level of society. Efforts made by the government for people with mental disorders began to
be implemented in the 2019 general election. This is the start of a change in perspective that not all people with mental disorders cannot participate in the general election. Efforts to change this point of view are based on 4 things that are always stigmatized in people with mental disorders, including equation of mental disorders with crazy people; concern about acts of vandalism that may be committed by people with mental disorders on voting day; concern that voices made by people with mental disorders can be manipulated and misused; and doubts about the capabilities of the mentally impaired at the time of voting.

In the eyes of the law, people with psychiatric disorders have the same rights as citizens who are not affected. Giving special treatment for sufferers of mental disorders is the maximum effort for respecting, advancing and fulfilling human rights for them. Contrary to this, the opinion that developed in the community later said that people with mental disorders are considered not to have the capability to make legal decisions, and it is even considered dangerous if a person with mental disorders continues to make legal decisions.

Based on data collected by the Ministry of Health, it is stated that the number of people with mental disorders in Indonesia reaches 7% of the total population of Indonesia as a whole. In the 2019 general election alone, the number of people with mental disorders who are counted in the DPT is 3,500 people. In accordance with the principle of the presumption of liberty which generally applies within the scope of human rights, it is the state that is burdened with the responsibility of being able to guarantee that every law that is enacted can accommodate all citizens and does not limit their movement. The state must be able to provide guarantees for people with mental disorders, in this case non-permanent mental disorders, to continue to have access to their constitutional rights.

Sufferers of psychiatric disorders are proven to have sufficient knowledge, a sense of responsibility for their actions, and the majority of them have positive feelings about the implementation of general elections. This explains that in the concept of democracy and rule of law, every vote that comes from a citizen cannot be reduced in any way. This is in line with the main pillars of the establishment of a rule of law, one of the pillars in order to create a strong rule of law requires guarantees of equal treatment before the law, protection of human rights, and social control.

Looking at the categorization of people with psychiatric disorders, there are several differences between someone who is said to be legally competent and someone who is psychiatrically unhealthy. The word refers to the legal situation faced by each sufferer. This in turn causes the person to be considered legally competent. Law focuses on the thoughts and mindset of a person who commits legal action, not looking at the organs that cause interference to a person who commits legal action. If a person is said to be legally competent in taking a legal action, then that person is still legally able to take legal action.

The Commission has directed that persons with mental disabilities to be included in the DPT. The KPU noted that there were 43,769 people with mental disabilities who had the right to vote in the 2019 elections. This number is expected to increase because data collection is still ongoing. In the 2014 election, there were only 8,717 voters with mental disabilities, while in the 2019 election it reached 43,769 people.

**Conclusion**

The implementation of inclusive elections is a very important part if the state wants to protect the right to vote for people with psychiatric disorders to participate actively in elections. Inclusive elections not only provide opportunities for persons with disabilities in general, but also pay attention to the needs of those who are also Indonesian citizens. Providing access to people with mental disorders will open up access for them in the policy making process, so that it will be possible for them to form policies that are more inclusive and friendly. Election inclusiveness can be the first step to improve the overall level of life of persons with disabilities. The right to vote for people with mental disorders is a right inherent in them. In general, the right to vote protected by laws and regulations applies to all Indonesian citizens. This then brings a consequence that people with mental disorders are one of the legal subjects whose voting rights must be protected.

**Ethical Clearance:** This research was ethically approved by Faculty of Law, Universitas Diponegoro,
Semarang, Indonesia.

**Funding:** This research receives funding of research grant from Faculty of Law, Universitas Diponegoro, Semarang, Indonesia.

**Conflict of Interests:** There are no conflict of interests

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Purified Pyocyanin from Clinical Isolates of Pseudomonas aeruginosa Enhances Antibiotic Sensitivity Against Some Pathogenic Bacteria

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Abstract

Pseudomonas aeruginosa is one of the most life-threatening pathogen. It is considered nosocomial opportunistic microbe that cause wide range of infections including wound and burn infections, respiratory infections, and Otitis media. Despite the efficiency of antibiotics against infectious diseases, P. aeruginosa still causes complicated infections with antibiotic resistance in many clinical strains. The pigments produced by P. aeruginosa exhibits antibacterial properties. Thus, we have examined its ability to enhance antibiotics effect against some pathogenic microbes. 286 samples were collected from patients with different infections who visited Mosul hospitals. 76 samples were positive to P. aeruginosa. Among them, 38 (13.28%) of isolates were isolated from surgical infection, whereas, 12 (4.19%), 11(3.84%), and 7(2.447%) were isolated from Otitis media, Urinary tract infection, and pus, respectively. The pyocyanin in low concentrations showed synergistic effect with some antibiotics against Staphylococcus aureus and Escherichia coli. E. coli became sensitive to ciprofloxacin and nalidixic acid when mixed with 100 mg/ml pyocyanin. However, cloxacillin did not show any activity against Staph.aureus when mixed with 1 mg/ml and 6.25 mg/ml pyocyanin. Staph aureus became sensitive to nalidixic acid when mixed with 1 mg/ml pyocyanin.

Key word: Pseudomonas aeruginosa, pyocyanin, Antibiotics, Synergistic effect.

Introduction

Pseudomonas aeruginosa is widely spread in the environment. It is a gram negative, aerobic microbe in rod shape belongs to Pseudomonadaceae family. In this family, there are 12 other genus as well as P. aeruginosa. Although P. aeruginosa can be found in soil and water, it is considered the most opportunistic pathogen in hospitals ¹⁹. It is also one of the nosocomial pathogens with antibiotic resistance strains ¹³ P. aeruginosa composed of virulence factors including polysaccharide layer, exotoxins, pilli and biofilm formation ability. The key factor of pseudomonal infections is bacterial attachment to the host epithelial layer that keeps the microbe away from the host immune defense ²¹.

P. aeruginosa produces many pigments, including pyocyanin (bluish green), pyoverdin (fluorescent yellow-green) and pyorubin (brownish red) ²⁸. These pigments contribute to pseudomonal infections. Many studies suggest that pseudomonal pigments can be a virulence factor in the lungs of patients with cystic fibrosis. They also may interfere host cell respiration. The mechanism of the pigment pathology is still unknown ²⁴.

The produced pyocyanin has antibacterial properties. It has been proved that pyocyanin prevents other bacterial growth is the site of infection ¹. This characteristic provides a persistence growth for P. aeruginosa. Many studies examined the possible activity of pyocyanin against other pathogens.

Multi-resistant strains of P. aeruginosa as nosocomial pathogen has spread in hospitals leading scientists to dig out other efficient drugs. In this study, we hypothesized that pyocyanin is a drug nominate and
whether can be used to enhance antibiotic efficiency by examining the synergistic effect of pyocyanin and antibiotics with less effect. We isolated and diagnosed clinical isolations from patients with different infections including otitis media, wound infection, surgical infection, urinary tract infection and upper respiratory infection. The synergistic effect of pyocyanin with antibiotics was examined.

**Materials and Methods**

**Samples collection and identification:**

286 samples were collected from different infection sites including wounds, otitis media, burns, surgical infections, urea and sputum, from patients who visited Mosul hospitals. Identification process were initially based on bacterial colony appearance on blood agar as well as the smell of culture. The haemolysis of blood on blood agar were reported as well as pigment production. Other standard diagnostic methods were performed according to. Molecular diagnosis of isolates were performed using 16S rRNA sequencing method. The genomic DNA were extracted using the extraction kit supplied from Geneaid. The genomic DNA were then visualized on agarose gel electrophoresis. The PCR reaction was carried out using the primers 16S RNA-F: AGAGTTTGATCCTGGCTCAG, and 16S RNA-R: AAGGAGGTGATCCAGCCGCA. The reaction was prepared by mixing 50 ng of DNA template with 10 pmol of primer mixture and 10 µl of hot start taq polymerase. The reaction was then topped up to 20 µl. The tubes were then put into the thermocycler and the program was run. The reaction condition was performed as 1 cycle of: initial denaturation at 95°C for 6 minutes, and 35 cycles of: denaturation at 95°C for 45 seconds, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute, and 1 cycle of: final extension at 72°C for 5 minutes. The amplicons were then visualized on agarose gel electrophoresis. The amplicons were then purified from agarose gel and extracted using DNA extraction kit. The purified PCR products were sent for sequencing using 16S RNA-F and 16S RNA-R. The sequence process was performed at Hitachi Company (Japan). The sequencer 3130 provided from Macrogen Biotechnology Company were used for gene sequencing. The sequence results were then blasted with DNA database using National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Pyocyanin extraction and purification:**

*P. aeruginosa* isolates were grown on King A media to confirm the pigment production and they were then grown on alanine minimal agar glycerol medium to enhance the pigment production. The cultures were incubated at 37° C for 48 hours. The culture were then exposed to light source with incubation at (25° C)°. The extraction of pyocyanin were performed according to the method described by Watson et al., 1986 with some modifications.

**Antibiotic susceptibility test:**

The sensitivity test was performed according to Clinical and Laboratory Standards Institutes (CLSI). Fresh bacterial culture were spread finely on Muller Hinton agar and left to dry. The antibiotic discs were put on the inoculated plates and incubated at 37 ° C for 24 hours. The antibiotics used in this study were penicillin, Cefixime, peperacillin, nalidixic acid, gentamycin, azithromycin, amikacin, ciprofloxacin, doxycycline, cloxacillin, erythromycin, chloramphenicol, amoxicillin, novobiocin, and tetracycline.

**Synergetic effect of pyocyanin with some antibiotics:**

The synergistic effect of pyocyanin with some antibiotics was performed according to the method described in with some modifications. The disc diffusion assay was used in this study. The optimal and minimal concentration of pyocyanin and antibiotics were reported against *Staph. aureus* and *E. coli*. The minimal concentrations of the pigment and antibiotics were then mixed to examine the synergistic effect of pseudomonal pyocyanin with antibiotics.

**Statistical Analysis**

Graphpad Prism software (Graphpad, California, USA) was used for statistical analysis. Means and standard error of means (SEM) were used to analyze the results. Significant difference was assessed at *p* values: * p<0.05, ** p<0.01.

**Results and Discussions**

Collecting of sample:
Among 286 samples collected from patients visited Mosul hospitals, we have isolated 76 positive samples of *P. aeruginosa*. The positive samples were confirmed using biochemical tests as well as molecular identification using 16S RNA gene sequencing. All samples tested for 16S RNA showed conserved sequence of the gene confirming that the gene is present in all *P. aeruginosa* isolates. The Figure 1 shows agarose gel electrophoresis of *P. aeruginosa* genomic DNA. This shows the purity and integrity of genomic DNA as the key factor of PCR process success is the purity of DNA used\(^{27}\). The Figure 2 shows agarose gel electrophoresis of PCR amplicons showing the exact size of gene of interest using the specific primers (16S RNA-F and 16S RNA-R). The amplicons were then purified from the gel and sent for sequencing. The results of sequence were then blasted and aligned with universal gene bank (NCBI). The molecular identification of microorganisms has been extensively used to confirm the bacterial identification. The gene polymorphism analysis is based on conserved sequences present in some housekeeping genes such as 16S RNA\(^3\). Although it is widely known that *P. aeruginosa* belongs to genetically diverse group of bacteria, 16S RNA gene is highly conserved in most of species with minor modifications\(^{30}\).

![Figure 1: Aggarose gel electrophoresis of *P. aeruginosa* genomic DNA.](image1)

![Figure 2: Aggarose gel electrophoresis of 16S RNA amplicons with expected size of 650bp DNA. 16S RNA-F and 16S RNA-R primers used to amplify the gene of interest. Lane 1: 100bp DNA ladder, lanes 2-9: DNA amplicons of gene of interest.](image2)
Table 1 shows the samples taken and the site of infection as well as the proportion of infections. As can be seen from the Table 1, the highest percentage of infection was 13.28% from surgery infections, whereas the lowest percentage was 0.699% from patients with burns. Surprisingly, *P. aeruginosa* isolated from the respiratory tract infections was relatively low (2.09%) compared to other site of infections. Although many studies have founded that *P. aeruginosa* is a main cause of burn infections with multidrug resistance \(^{16,21}\), we found that the burn infections was the lowest. This might be because of the extent of the hospital’s concerns with the cleanliness of the hallways and medical equipment as well as the type of detergents and disinfectants that used in hospitals \(^{15}\). It has also been reported that *P. aeruginosa* favors the moist and aerobic conditions to cause infections \(^{23}\). This explains the reason of the variation of *P. aeruginosa* infections might be affected by the site of infection and environment conditions.

**Table (1):** The number and percentages of the total samples of *Pseudomonas aeruginosa* isolated from different sites of infection.

<table>
<thead>
<tr>
<th>Swab site</th>
<th>No. of samples</th>
<th>No. of <em>P. aeruginosa</em></th>
<th>% for each site</th>
<th>% relative to total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otitis media</td>
<td>17</td>
<td>12</td>
<td>70.58%</td>
<td>4.195%</td>
</tr>
<tr>
<td>Burns</td>
<td>2</td>
<td>2</td>
<td>100%</td>
<td>0.699%</td>
</tr>
<tr>
<td>Surgical infections</td>
<td>55</td>
<td>38</td>
<td>69.09%</td>
<td>13.28%</td>
</tr>
<tr>
<td>Sputum</td>
<td>61</td>
<td>6</td>
<td>9.83%</td>
<td>2.09%</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>123</td>
<td>11</td>
<td>8.94%</td>
<td>3.84%</td>
</tr>
<tr>
<td>Wound infections</td>
<td>28</td>
<td>7</td>
<td>25%</td>
<td>2.44%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>286</strong></td>
<td><strong>76</strong></td>
<td><strong>----------------</strong></td>
<td><strong>26.57%</strong></td>
</tr>
</tbody>
</table>

**Synergistic effect of pyocyanin and antibiotics:**

In order to examine the activity of pyocyanin with antibiotics, the optimal concentration of antibiotics that did not show activity against *Staph. aureus* and *E. coli*. Antibiotic resistance test showed diverse activity of antibiotics used in this study. The Table 2 shows antibiotic activity on *Staph. aureus* and *E. coli*. As can be seen from the Table 2, the *Staph. aureus* showed resistance against most antibiotics used. Therefore, we have chosen cloxacillin and novobiocin that showed either intermediate or resistance. Regarding *E. coli*, the results also showed that the microbe was resistant to most antibiotics (Table 2). The antibiotics have chosen for synergistic activity for *E. coli* were ciprofloxacinc and nalidixic acid.

**Table (2):** Antibiotics resistance test showing the effect of antibiotics on *Staph*. *aureus* and *E. coli*. The antibiotics have chosen according to \(^{5}\) protocol. *: Sensitive; **: Resistant; ***: Intermediate.

<table>
<thead>
<tr>
<th>n.</th>
<th>antibiotics</th>
<th>code</th>
<th>Result</th>
<th>antibiotics</th>
<th>code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Penicillin</td>
<td>P</td>
<td>S*</td>
<td>Doxycycline</td>
<td>DO.10</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>Cefixime</td>
<td>CFM</td>
<td>S</td>
<td>Cloxacillin</td>
<td>CX.10</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>Piperacillin</td>
<td>PRL</td>
<td>S</td>
<td>Erythromycin</td>
<td>E-10</td>
<td>R</td>
</tr>
</tbody>
</table>
Pyocyanin was extracted and purified from the culture to examine its activity against the pathogens used in this study. Different concentrations of pyocyanin used against Staph. aureus and E. coli to choose the concentrations that have either intermediate or no activity. The reason for choosing these concentrations to find out whether pyocyanin could enhance antibiotics activity. The results showed that P. aeruginosa produced pyocyanin in different concentrations (Table 3). The production of pyocyanin is influenced by many factors including environmental and genetic factors. It has also been reported that the site of infection might affect the amount of pyocyanin produced by P. aeruginosa. The production of the pigment might also be affected by other pathogens at the same site of infection. It is proved that P. aeruginosa produces high concentrations of pyocyanin to compete other pathogens as the pigment has antibacterial properties. Although all strains of P. aeruginosa have genes that encode to pyocyanin, the expression level of these genes might be influenced by environmental conditions and whether the pathogen requires the pigment during the infection.

Table (3): The percentage of pyocyanin extracted from 10 strains of P. aeruginosa. The strains were named as Du to refer to the researchers name.

<table>
<thead>
<tr>
<th>n</th>
<th>Strain</th>
<th>Concentration mg / 25 ml</th>
<th>percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Du1</td>
<td>0.207</td>
<td>0.828%</td>
</tr>
<tr>
<td>2</td>
<td>Du2</td>
<td>0.312</td>
<td>1.248%</td>
</tr>
<tr>
<td>3</td>
<td>Du3</td>
<td>0.370</td>
<td>1.48%</td>
</tr>
<tr>
<td>4</td>
<td>Du4</td>
<td>0.152</td>
<td>0.608%</td>
</tr>
<tr>
<td>5</td>
<td>Du5</td>
<td>0.340</td>
<td>1.36%</td>
</tr>
<tr>
<td>6</td>
<td>Du6</td>
<td>0.244</td>
<td>0.976%</td>
</tr>
<tr>
<td>7</td>
<td>Du7</td>
<td>0.248</td>
<td>0.992%</td>
</tr>
<tr>
<td>8</td>
<td>Du8</td>
<td>0.236</td>
<td>0.944%</td>
</tr>
<tr>
<td>9</td>
<td>Du9</td>
<td>0.235</td>
<td>0.94%</td>
</tr>
<tr>
<td>10</td>
<td>Du10</td>
<td>0.305</td>
<td>1.22%</td>
</tr>
</tbody>
</table>
Synergistic activity showed that *E. coli* became more sensitive to antibiotics when mixed with pyocyanin. *Staph aureus* showed intermediate resistance when antibiotics mixed with pyocyanin. The Figures 3 and 4 shows synergistic activity of pyocyanin with antibiotics against *E. coli* and *Staph. aureus*. As can be seen from the Figure 3, although *E. coli* was intermediate resistant to ciprofloxacin and resistant to nalidixic acid, it became sensitive to the antibiotics when mixed with 100 mg/ml pyocyanin (Table 4). However, ciprofloxacin did not show any activity against *E. coli* when mixed with 10 mg/ml pyocyanin (Figure 3, Table 4). This might be because pyocyanin activity is concentration dependent manner when mixed with antibiotics. Regarding *Staph. aureus*, the results showed that the pathogen became sensitive to novobiocin when mixed with 1 mg/ml pyocyanin (Figure 4, Table 4). It is possible that pyocyanin enhanced the activity of novobiocin in terms of DNA gyrase inhibition. Other explanation might be that pyocyanin might interact with bacterial DNA causing mutations that in favor to novobiocin activity 12, 29. However, Cloxacillin did not show any activity when mixed with 1 mg/ml and 6.25 mg/ml pyocyanin, respectively (Figure 4). The limit activity of antibiotics with *P. aeruginosa* pigment reflects the fact that Gram positive bacteria might exhibit resistance strategies against antibiotics, and thus, pyocyanin could not enhance the activity 9.

It is reported that the ability of inhibition of pyocyanin is increased over the years as a result of the ability of bacteria to adapt to the environment and produce more efficient virulence factors. This might be due to the development of their genes that encode to pyocyanin more efficiently 25. The synergistic activity of pyocyanin with antibiotics might be affected by the cell wall composition. It is clear that peptidoglycan and lipopolysaccharides layer could prevent the penetration of antibiotics as well as pyocyanin 7,30,31. This explains why *Staph. aureus* is less affected by pyocyanin and antibiotics as the thickness peptidoglycan layer reduces the penetration. On the other hand, *E.coli* showed less sensitivity against pyocyanin and antibiotics. The peptidoglycan layer in Gram negative pathogens is not thick compared to Gram positive bacteria 8,20. Cloxacillin belongs to penicillin antibiotic family that can be broken down by staphylococcal beta-lactamase enzymes 11. These enzymes might affect the synergistic activity of cloxacillin with pyocyanin, and thus, did not show any activity.

It is widely known that pyocyanin stimulates the formation of free radicals (-O₂ and H₂O₂). This leads to increase the oxidative stress in bacterial cells and makes them more affected by antibiotics 26. It is also reported that generation of active oxygen compounds (ROS) due to the exposure to pyocyanin stops all NADPH pathways by excluding electron 12. The gene expression is also affected by ROS and this might be beneficial for antibiotics to stop protein synthesis of bacterial cells 4. Therefore, pyocyanin activity against pathogens could be beneficial to test whether can be used in antibiotics structure to boost their activity.

Table (4): Synergistic effects of pyocyanin with antibiotics. The concentration of pyocyanin were chosen as they have shown no effect on *Staph. aureus* and *E. coli*. Antibiotics used in this test were chosen because they did not show activity against pathogens.

<table>
<thead>
<tr>
<th>Antibiotics activity</th>
<th>Staph.aureus Pyocyanin activity</th>
<th>Synergistic activity</th>
<th>E.coli Antibiotics activity</th>
<th>Pyocyanin activity</th>
<th>Synergistic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX.10</td>
<td>I</td>
<td>1mg/ml</td>
<td>I</td>
<td>R</td>
<td>CIR</td>
</tr>
<tr>
<td>NV</td>
<td>R</td>
<td>1mg/ml</td>
<td>I</td>
<td>S</td>
<td>NA</td>
</tr>
<tr>
<td>CX.10</td>
<td>I</td>
<td>6.25mg/ml</td>
<td>R</td>
<td>R</td>
<td>CIR</td>
</tr>
</tbody>
</table>
Ethical statement: The project is done according to the ethical standards of Iraqi medical institutions. Ministry of Medicine, Iraq.

Source of Funding: This study was funded by the authors. All article preparation process and publication has done by authors and no other sources involved.

Conflict of Interest: Nil

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Efficacy of Plyometric Training on the Agility in Police Cadets

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Abstract

Background: Plyometric training is a hybrid between strength and endurance training. This allows muscles to exert maximum force in short intervals of time, with the goal of increasing power (speed-strength) which ensures better agility in police cadets. Agility has been defined by the capacity to retain or determine the location of body by shifting its direction rapidly in a sequence of movements. Agility, speed and explosive power are qualifying components of physical fitness and desirable athletic performance, and play a key role in most sports. Agility can enhance the coordination and regulation of locations of the body throughout movement.

Objective: To study the efficacy of the 6-weeks and 12-weeks Plyometric training on the agility in police cadets.

Method: 40 Cadets aged above 18 years were grouped into two. A group continued their regular activities while rest underwent 2 sessions of plyometric training every week for 6 weeks, along with their daily activities. Analysis was then carried out with assessment of T-test Agility test, Illinois Agility Test, Edgren Side Step Test. Study duration is 6 months and intervention duration is 12-weeks, hence participants will be enrolled during first 3-months of study so 12 week intervention will be completed successfully. Assessment will be done on 1st day of visit then at the end of 6th week and again at the end of 12th week. Participants would have to perform 2 session of Plyometric Training per week in other group.

Result: Data will be analyzed using paired T-test.

Conclusion: Will be published after the results are analyzed.

Key words: Plyometric training, Agility, Agility Test.

Introduction

For the physical preparation of the job, tactical sportsmen, such as military, firefighters, or police officers require speed, strength, agility and stamina training.

While research has shown that the role of a police officer is shockingly often sedentary, it is recognized that physical fitness is an important component of being able to perform unusual and often vital tasks, such as chasing running people, managing arrest and handcuffs and even managing crowds. A police officer’s capacity to perform these various physical tasks will decide his professional performance. The tactical athlete literature tends to be a void with a strong emphasis on the physical qualifications of the Police Cadet and the outcomes of training within the academy.¹ Police officers are expected to perform a range of physical activities, including housekeeping and checking the identity of a suspect to chasing suspects on foot over different distances.
Plyometrics are conditioning methods that athletes use to boost strength and quickness in all kinds of sports. Plyometrics is composed of a rapid muscle lengthening followed right away by shortening movement of the muscle and tissue which are connective. Greater strength is generated than the concentrated action itself by the collected elastic energy inside the muscle.

Researchers have explained about plyometric exercises which can lead to changes in efficiency in jumping vertically, agility, strength of leg and that of muscles, improved perception of articulation of bones and muscular sense in whole with the use of a periodic strength training plan. Plyometric drills usually involve the explosive stop, start, and change of direction. All such movements are elements that can help the agility to develop.

Plyometric exercises — jump, striding, and hop movements which use the stretch muscle unit to alter the process. — It has repeatedly been shown to boost muscular strength and power generation. It is used to improve maximum strength, movement speed and explosive power increase. Dynamic in nature, these exercises comply with the basic training concepts of precision, practice with movements of a similar nature and pace to the skills or activities for which one is trained.

In fact, the rapid force efficiency of the training muscle is increasing, combined with smaller rises in isometric force level. Such physiological improvements have allowed higher vertical jump heights and reduced sprint and acceleration times. Agility has been defined by the capacity to retain or determine the location of body by shifting its direction rapidly in a sequence of movements. Agility helped in motor learning via neuromuscular stimulation and adaptation of neural spindle, golgi organs of tendon, and proprioceptors present in joint. Theoretically, agility can enhance the coordination and regulation of locations of the body throughout movement.

Agility, speed and explosiveness are the characteristics of physical fitness and play a important role in most sports. Agility components includes speed, balance, strength and coordination; necessary for technical, competitive sports and “tactical” players in order to change position quickly for sport or work on all planes. Upgraded agility benefits involve improved body flexibility throughout rapid movement, improved intramuscular mobility and reduced risk of injury or re-injury. Agility skills will require a strong mix of dynamics. The balance system will have to be calibrated and modified because the fast code would move the centre of gravity out of the base of support again and again and challenge balance or metastability.

Michael G. Miller et.al determined that plyometric training course of 6 weeks influences the agility and inferred that it has encouraging effect and benefits in increasing the agility in athletes.

Kevin Thomas and team determined results of 2-plyometric conditioning technique over strength of muscle in young soccer players and results determine both Counter Movement Jump and Depth Jump. Plyometric Training are useful in training exercises to develop strength and agility among young soccer players.

Issam Makhlouf et.al explained that strength and plyometric training together has equal fitness advantage with the combination of endurance and plyometric training of soccer players of younger age group and the result are significant improvements in agility in both training group of soccer players.

Although it has been shown that plyometric training enhances performance variables, i.e. agility training targets for visual orientation, retain a good position yet little empirical evidence is present to establish whether the plyometric training actually increases agility.

Objectives
1. To determine the effect of 6-week plyometric training program on agility of the cadet.
2. To determine the effect of 12-week plyometric training program on agility of the cadet.

Methods
This study will be carried out at training camps which are in the vicinity of Wardha District, Wardha, Maharashtra, India only after approval by the Datta Meghe Institute of Medical Sciences Institutional Ethics Committee, Deemed University.
Study design: Experimental Study

Study duration: 6 months.

Inclusion Criteria: -
1. Participants over 18 years of age.
2. Free from lower extremity injuries.
3. At the time of the analysis, they did not engage in any form of plyometric training.

Exclusion Criteria: -
1. History of injuries to the lower limb in recent times.
2. Fractures in the lower limb in near past.
4. Non-cooperative participants.

VARIABLES

Outcome measures:
1. T-test Agility test
2. Illinois Agility Test
3. Edgren Side Step Test

DATA SOURCE MEASUREMENT

1. T-test Agility test - Agility is measured with r=0.98, p<0.05.\(^7\) that designates the reliability and validity of the T-test.

2. Illinois Agility Test – It is a reliable and valid velocity change tool with r=0.77, p<0.001.\(^8\)

3. Edgren Side Step Test- Another valid as well as reliable tool for changing in duration and speed with r=-0.640, p = 0.046.\(^9\)

STUDY SIZE –

Group A: 20 participants will continue their regular activities.

Group B: 20 participants must undergo 2 sessions of plyometric training every week for 6 weeks, along with their daily activities.

Procedure

The institutional ethics committee clearance will be obtained before the start of the study. The permission will be obtained from the head of institute for cadets and after meeting the criteria for inclusion and exclusion, the informed consent must be received from the participants. Participants are classified into two categories, i.e. Plyometric Training Group and Control Group. Several tests were developed in order to measure agility, but few were defined for young adult males as effective or legitimate measures and no connection between the tests was established.\(^5\) Demonstration of agility tests would be given to both groups. And plyometric training would be demonstration only to experimental group.

During the time period of training, all participants will be told not to alter their current physical activities. The participants in plyometric training group will receive plyometric training for 6 week which consists of range of plyometric exercise designed for the lower extremity, whereas no plyometric exercises will be performed by the control group. The plyometric exercise is Squat jump, tuck jump, lateral jump, lunge jump etc. During the 12\(^{th}\) -week’s duration, all subjects will continue normal daily living activities. The Plyometric training program consists of 2 training programs in a week. The training depends on intensity as well as volume, using similar exercises, sets, and replays.\(^2\)

Pre and Post 6\(^{th}\) week and 12\(^{th}\) week-training assessments will be used to analysis the agility results. The T-test gives the measure of the speed of variation in direction. The Illinois agility test assesses the preparedness to increase, decrease, rotate in various directions and move in various angles.

T-Test Procedure

Describe the participant regarding test procedures. Conduct health risk screening and gain informed consent. Prepare forms and record basic details such as age, height, weight, gender, test conditions. Measure and classify the test area. Carry out a good warm-up. Five meters apart three cones are placed in a straight line. 10 meters from the middle cone another cone is positioned and a ‘T’ shape is formed. Start point of cadets is the base of the ‘T’. Go signal is given by the examiner and the period starts as the cadets crosses the mark point.
Cadet heads to the centre cone and hits it. Cadet runs 5 metres towards cone at the right and hits it. Cadet’s hand moves 10 meters to the far cone and touches that one. Cadet’s hand moves back to the middle cone for 5 meters and hits it. Cadet moves 10m backwards and touches the cone at the ‘T’ base. When the participant reaches the mark point, the time is stopped and duration is measured.

Illinois Agility Test procedures

Describe the participant regarding test procedures. Conduct health risk screening and gain informed consent. Prepare forms and record basic details such as age, height, weight, gender, test conditions. Measure and classify the test area. Carry out a good warm-up. The test has agility region (10 metres length and 5 meters breadth) that is formed by four cones. Point A cone marks the start point. The turning point is marked by Cone B&C. The end point is shown by Cone D. At the centre of the test area, four cones are positioned 3.3 meters apart. Starts with the person lying down and facing the ground and hands on his side. “Go” button signals the cadet to begin and as the start marker point is crossed, the time starts. The cadet is asked to get up and run in the course of the direction chosen (left to right / right to left) by him. At the turn points B and C, cones are supposed to be touched by hands. As the cadet reaches the finish line and no cones are knocked over and duration is assessed which completes the trial.

Edgren Side Step Test

Describe the participant regarding test procedures. Conduct health risk screening and gain informed consent. Prepare forms and record basic details such as age, height, weight, gender, test conditions. Measure and classify the test area. Carry out a good warm-up. The course is 4 m long and has four intervals of 1 m. The beginning position is at the centre of the circle, facing forward, with the legs straddling the centre line. With the ‘go’ signal, the person moves in right direction before the right foot crosses the outer cone. The participant then moves in the left direction before his left foot hits the left outer cone. The person runs in backward and forward direction to the outer cones as quickly in duration of 10 seconds. A score of 0 is given to the cadet, if he had not kept the trunk and legs pointing forward in the test period, legs crossed, or failed to successfully complete the course.

After recording the results of the agility test at the beginning of the plyometric group training, the plyometric training process will be clarified and again the agility test results will be reported after the 6th week of training and the 12th week of training for both classes.

Expected Results

After completion of the study result will be calculated by statistical analysis using paired T-test and will be presented in the form of research paper.

Discussion

To our knowledge, this will be the first study to evaluate the effects of plyometric training on the aspect of agility in the Indian population of police cadets. This is important as good reporting of how agility training is going to impact the agility specific outcomes. Also the process of involving agility training into the regular strength training programs of these cadets would change the focus of just strength training in their routines. More over this study will employ well established and widely used methods with appropriate reliability and validity to assess the agility parameters. Post the sessions at the end of 6th week assessments will provide a immediate effect of the agility training and another timeline of assessments after total 12 weeks will provide the differences to have better understanding about the improvements sustained. The potential lack of keeping a track on the cadet’s activities between sixth to twelfth weeks may represent a limitation of the study. Although we assume that the cadets would have a better understanding towards agility-based training in regular routines.

References

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Correlation between CD4 T lymphocyte and Candida Species Counts In Oral Candidiasis Patients with HIV / AIDS

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Abstract

Background: Candida sp. is the most common opportunistic pathogen found during the development of Human immunodeficiency virus (HIV) & Acquired immune deficiency syndrome (AIDS) disease. The clinical severity of oral candidiasis and the prevalence profile of Candida species reflect immunological changes in HIV / AIDS patients. Objective: Evaluating the relationship between CD4 T lymphocyte cells counts and the number of Candida species. Methods: A cross-sectional analytical study was carried out at Dr. Soetomo, Surabaya, Indonesia. For identification of Candida species, culture was carried out on Chromagar media followed by culture with vitek 2. Results: There were 114 study subjects who were divided into three groups based on the number of CD4 T lymphocyte cells, with 158 isolates of Candida species growing in culture. The highest number of Candida species was Candida albicans with a total of 107 isolates (67.7%). Candida non-albicans were 51 isolates (32.3%). Statistical test results showed a significant correlation between the number of CD4 T lymphocyte cells and the number of Candida species (p <0.001). Conclusion: The decrease in CD4 lymphocyte cell counts is influenced by various types of Candida sp. in oral candidiasis patients.

Keywords: Candida sp, CD4 T lymphocytes, HIV / AIDS, oral candidiasis

Introduction

In the human oral cavity, there are various kinds of microorganisms which composition, metabolism activities and pathogenicity are influenced by external and internal factors. Among all fungi, Candida is a genus of yeast that is considered to be the most fungal species found in the oral cavity. Candida species are the main cause of mucocutaneous infections which are usually classified as oral, oesophageal and vulvovaginal candidiasis (1, 2).

Oral candidiasis (OC) is the most common mucocutaneous candidiasis found in patients with infection of Human Immunodeficiency Virus (HIV) / Acquired Immune Deficiency Syndrome (AIDS) worldwide. OC is an infection of the mucosa caused by Candida species. Nearly 90% of patients with HIV / AIDS have experienced OC in the course of their illness. High viral load and low CD4 T lymphocyte counts are found in OC patients (1, 2). Although oral candidiasis is caused by various genera of Candida, Candida albicans is the most common cause as stated by previous studies, but several studies found an increase in the cause of oral candidiasis due to Candida non-albicans (3, 4). This study aimed to analyse the relationship between CD4 cell counts and Candida species in oral candidiasis patients with HIV / AIDS.
Methods

Participants

Participants in this study were HIV / AIDS patients at Dr. Soetomo General Hospital Surabaya, Indonesia. Participant inclusion criteria included patients diagnosed with HIV / AIDS using a rapid test / HIV 3 \(^5, 6\), having a positive candidiasis diagnosis using 10-20% KOH \(^7, 8\), and aged >18 years. Participant exclusion criteria included subjects taking anti-fungal drugs within 2 weeks before the study took place and no growth of colonies was found on culture examination. Participants in this study first filled out the consent form, in which the patient had received an explanation regarding the benefits and objectives of the study.

Design

This research was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from May to September 2019. It was a cross-sectional study with consecutive admission sampling method. There were 114 participants, which were divided into 3 groups (38 participants in each group; Figure 1). This study was ethically approved by the ethics committee of Dr. Soetomo General Hospital (1125 / KEPK / IV / 2019). The research procedure consisted of identifying the type and amount of \textit{Candida} fungus in the participant’s oral cavity and calculating the participant’s CD4 count.

Measurement of \textit{Candida} Species

Participants were first told not to do oral hygiene before checking the type and amount of colonisation of \textit{Candida} sp. Participants gargled using 25 ml of sterile water. The water was then put into sterile bottles that had been prepared. Water from the mouth rinse was immediately sent to Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The water was shaken gently, and took ± 5 - 10 ml and put in 15 ml sterile centrifuge tubes and rotate 2,500 revolutions per minute (rpm) for 5 minutes. Discard the supernatant and take the pellet using a sterile pipette, place it in an object glass and drop 10-20% KOH. \textit{Candida} positive was planted on Sabouraud dextrose agar (SDA) at 37ºC for 48 hours \(^9\). The SDA media used was CHROMagar Candida (CHROMagar Candida, France). Growing \textit{Candida} specimens were identified using cornmeal agar and tween 80 incubated at 42-45ºC \(^{10, 11}\).

CD4 Measurement

The researchers took venous blood samples and put into K3EDTA-countaining tubes, stored at room temperature, immunophenotyping for 6 hours. CD4 count calculations used flow cytometry (BD FACSCounttm System; San Jose, CA). Participants were divided into three groups: group I (participant with a CD4 level of \(\leq 100 / \mu \text{L}\)), group II (participant with a CD4 level of 101 - 200 / \(\mu \text{L}\)), and group III (participant with a CD4 level of > 200 / \(\mu \text{L}\)).

Lesion Area

The area of participant’s candidiasis oral lesions was assessed using a score of 0 = no lesions, 1 = partial lesions, and 2 = all / all lesions. The locations assessed included the tongue, mucous membrane, and palate with a minimum score of 1 and a maximum score of 6. Calculation of the area of the lesion by adding up the three locations of the assessed lesion.

Statistical Analysis

The results of the study were presented in the form of mean ± standard deviation (SD) or median (minimum - maximum) and percentage (%). Statistic analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). Analysis of the measurement data used the Chi Square test, Fisher exact test, the Spearman correlation test, or the Anova test with a significance level of \(p <0.05\).

Results

The study showed that 114 oral candidiasis patients with HIV / AIDS met the inclusion criteria as research subjects. The age characteristics of the study subjects had a standard deviation of 36.41 ± 9.825 years, with an age range of 18 years to 59 years (Table 1). Most research subjects worked as private employees (58.8%). As many as 48.2% of research subjects had a high school equivalent education level (Table 2).
The clinical condition of oral candidiasis in this study showed the main complaint in form of white patches in the oral cavity that was found in 100 subjects (87.7%). Most locations were found on the tongue in 54 people (47.4%). Most participants were not accompanied by swallowing pain (64 participants; 56.1%). The most common clinical condition of OC was pseudomembranous type found in 103 patients (90.4%), followed by acute cheilitis (10 participants; 8.8%) and acute atrophic (1 participant; 0.9%) (Table 2).

The identification of species from 114 patients found 158 isolates of *Candida* sp., consisting of *Candida albicans* (107 isolates; 67.7%) and 51 non-albicans isolates (32.3%). The highest number of *Candida non-albicans* species were *Candida krusei* (26; 16.5%), *Candida glabrata* (13; 0.8%) and *Candida tropicalis* (7; 0.4%). This study also found rare *Candida* isolates, namely *Candida parapsilosis, Candida dubliniensis,* and *Candida lypolitica* (Figure 2). Spearman test obtained a significant relationship between the number of CD4 T lymphocyte cells with the number of species (p < 0.001), and a significant relationship between the number of species with lesion area (p = 0.004). However, the relationship was not significant between the number of species with duration of sickness (p = 0.081). Fisher Exact test showed no significant relationship between the number of CD4 T lymphocyte cells with clinical condition (p = 0.016), and a non-significant relationship between the number of species and clinical condition (p = 0.284; Table 3).

### Table 1. Demographic data of research subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83 (72.8)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (21.2)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>41 (36.0)</td>
</tr>
<tr>
<td>Public employee</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td>Private employee</td>
<td>67 (58.8)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
</tr>
<tr>
<td>Not completed elementary school</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Elementary school</td>
<td>20 (17.5)</td>
</tr>
<tr>
<td>Junior high school</td>
<td>21 (18.4)</td>
</tr>
<tr>
<td>Senior high school</td>
<td>55 (48.2)</td>
</tr>
<tr>
<td>University</td>
<td>17 (14.9)</td>
</tr>
</tbody>
</table>
Table 2. Clinical description of research subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General complaint</td>
<td></td>
</tr>
<tr>
<td>Red patches on the oral cavity, patches and sores on the corners of the lips</td>
<td>7 (6.1)</td>
</tr>
<tr>
<td>White and red patches on the oral cavity</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>White patches on the oral cavity</td>
<td>100 (87.7)</td>
</tr>
<tr>
<td>White patches on the oral cavity, patches and sores on the corners of the lips</td>
<td>3 (2.6)</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>50 (43.9)</td>
</tr>
<tr>
<td>No</td>
<td>64 (56.1)</td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Mix</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td>Tongue</td>
<td>54 (47.4)</td>
</tr>
<tr>
<td>Tongue, mix</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Tongue, mucosa, mix</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Tongue / mucosa</td>
<td>38 (33.3)</td>
</tr>
<tr>
<td>Tongue / mucosa / lips</td>
<td>11 (9.6)</td>
</tr>
<tr>
<td>Mucosa</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Clinical condition</td>
<td></td>
</tr>
<tr>
<td>Acute pseudomembrane</td>
<td>103 (90.4)</td>
</tr>
<tr>
<td>Acute atrophic</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Cheilitis</td>
<td>10 (8.8)</td>
</tr>
<tr>
<td>Chronic hyperplastic</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Table 3. Correlation between CD4 T lymphocyte counts and Candida species in oral candidiasis patients with HIV / AIDS

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of Species</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (%)</td>
<td>II (%)</td>
</tr>
<tr>
<td>CD 4 counts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤100</td>
<td>23 (60.5)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>101-200</td>
<td>23 (60.5)</td>
<td>15 (39.5)</td>
</tr>
<tr>
<td>&gt;200</td>
<td>37 (97.4)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Lesion Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>48 (57.8)</td>
<td>10 (38.5)</td>
</tr>
<tr>
<td>3-4</td>
<td>29 (34.9)</td>
<td>15 (57.7)</td>
</tr>
<tr>
<td>5-6</td>
<td>6 (7.2)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Duration of sickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 days</td>
<td>33 (39.8)</td>
<td>11 (42.3)</td>
</tr>
<tr>
<td>8-30 days</td>
<td>30 (36.1)</td>
<td>12 (46.2)</td>
</tr>
<tr>
<td>&gt;30 days</td>
<td>20 (24.1)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>Clinical Condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute pseudomembrane</td>
<td>77 (92.8)</td>
<td>22 (84.6)</td>
</tr>
<tr>
<td>Acute atrophic</td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cheilitis</td>
<td>5 (6.0)</td>
<td>4 (15.4)</td>
</tr>
</tbody>
</table>

Abbreviation: *significant p < 0.05; **significant p < 0.001
Figure 1. Flowchart Diagram of Participant

- HIV/AIDS patients (n=716)
  - Outpatients (n=572)
  - HIV/AIDS inpatient (n=144)
    - Other diseases (n=9):
      - Diabetes mellitus (4)
      - Kidney failure (1)
      - Tuberculosis (3)
      - Arteriosclerosis (1)
    - Oral Candidiasis (n=135)
      - No culture growth (n=21)
        - Oral Candidiasis with HIV/AIDS (n=114)

Figure 2. Prevalence of candida in Indonesian patient living with HIV/AIDS

C. lypolitica 1
C. dubliniensis 2
C. parapsilosis 2
C. tropicalis 7
C. glabrata 13
C. krusei 26
C. albicans 107

Figure 2. Prevalence of candida in Indonesian patient living with HIV/AIDS
Discussions

Based on gender, the distribution of subject was dominated by male patients (83; 72.8%) compared to female (31; 27.2%). This finding is consistent with data from the Indonesian Report of HIV Development Situation published by the Ministry of Health of the Republic of Indonesia in 2017, in which the pattern of HIV patients was more prevalent in male than female group (5). In general, there is no difference in terms of disease occurrence based on gender because unlike vulvovaginal candidiasis, oral candidiasis is not influenced by hormonal factors. Characteristic of subject’s age in this study had a standard deviation of 36.41 ± 9.82 years, with an age range of 17 years to 59 years. This age range is considered as productive age and sexually active, so that many may perform unprotected sexual behavior that is at risk of HIV transmission (4). In terms of distribution of educational levels, there were still research subjects who did not complete elementary school (1; 0.9%), and 20 subjects (17.5%) had elementary school education. In this study, there were still HIV/AIDS patients with low educational background, so that they had lack of oral health knowledge and a very poor ability to maintain oral hygiene. Poor oral cleaning is one of the factors that could increase the incidence of oral diseases, including oral candidiasis (5, 13). The distribution of occupational backgrounds showed that 41 (36%) patients did not work. Patients with HIV/AIDS in the course of their illness will experience opportunistic infectious diseases that will affect the quality of life, one of which is occupation (2, 4, 13).

The description of the main complaints from OC patients with HIV/AIDS in this study had more than one type. The most common complaint was white patches found in the oral cavity (100 subjects; 87.7%), followed by white and red patches in the oral cavity (4 subjects; 3.5%). Complaints of red patches, patches or sores on the corners of the lips were found in 7 subjects (6.1%), while white patches on the oral cavity, patches and sores on the corners of the lips were found in 3 subjects (2.6%). Complaints of white patches in the oral cavity were found in the pseudomembranous OC (38.3%) and plaque hyperplasia (9.6%). The subjects were mostly not accompanied by complaints of swallowing pain (64; 56.1%). Patients with complaints of swallowing are clinical signs of lesions attacking the pharynx and esophagus (14, 15).

Infection of Candida sp. is always associated with OC in HIV/AIDS. Candida sp. is a commensal microorganism that develops into pathogens due to a decrease in the secretion of immunoglobulin A (sIgA) and a decrease in the number of T lymphocyte cells (8). The pathogenesis of OC in HIV is closely related to proteins produced at the beginning of HIV virus replication cycle. Trans-Activating Transduction or Transcriptional Activator (TAT) is one of the proteins that regulates HIV virus replication. This protein can resemble the workings of extracellular matrix proteins (Integrin-like) in the regulation of cell life activities through activation of signal transduction pathways. The cell wall of C. albicans is thought to have receptors that resemble extracellular matrix (Integrin-like) which can bind to TAT. Interaction between HIV TAT protein with C. albicans cell wall allows the occurrence of specific gene transduction in candidiasis in HIV/AIDS with CD4 T cell lymphocyte counts <200 cells / mm (16, 17).

In this study, identification of Candida species conducted on 114 subjects found 158 isolates of Candida species with different species variants. Candida albicans was found in 107 isolates (67.7%), whereas Candida non-albicans was found in 51 isolates (32.3%). The highest number of Candida non-albicans species were Candida krusei (26; 16.5%), Candida glabrata (13; 0.8%) and Candida tropicalis (7; 0.4%). This study also found rare Candida isolates, namely Candida parapsilosis, Candida dubliniensis, and Candida lypolitica. This proves that in OC with HIV/AIDS, there has been a change in the spectrum of the causative species (12). In addition, the high detection rate of Candida non-albicans in this study is due previously incorrect identification of species due to the similarity of the phenotype to C. albicans and is now increasingly recognized. The proportion of Candida infections caused by C. albicans in HIV/AIDS patients has shifted to Candida non-albicans species, corresponding to previous studies comparing Candida species causing OC in HIV-seropositive patients and HIV-seronegative patients. The study showed that colonies of Candida albicans species in HIV-seropositive was less than in HIV-seronegative. In HIV-seropositive patients, Candida species are also rarely found in immunocompetent patients, namely Candida dubliniensis, Candida glabrata, Candida
A meta-analysis and study conducted in Sub-Saharan Africa in 2005-2015 showed a prevalence of *Candida non-albicans* species as much as 33.5% in general, with dominant species namely *Candida glabrata*, *Candida krusei*, and *Candida tropicalis* (19). The results of this study also showed a mixed infection between *Candida albicans* and *Candida non-albicans*. Out of 114 subjects in this study, there were 31 subjects (27.2%) with more than one *Candida* species isolate, and 83 subjects (72.8%) with one type of *Candida* species isolate. A meta-analysis study conducted in Sub-Saharan Africa for 10 years also showed the incidence of mixed infections of *Candida albicans* and *Candida non-albicans* by 85.2% (19, 20).

Oral candidiasis in HIV / AIDS patients is often of longer duration, repeated, and has more severe clinical symptoms. In HIV / AIDS patients, viral replication has the potential to trigger OC, which directly increases the progressive rate of HIV / AIDS infection. The Long Repeat Terminal (TLR) at the end of the provirus has two sites that function as transcription factors that bind the host, NFkB and SP1. Infected T cells are activated and release cytokines such as Interleukine-2 (IL-2) and Tumor Necrosis Factor (TNF). IL-2 and TNF will induce HIV provirus to end the latent period and start replicating. T cells will facilitate the replication of the HIV / AIDS virus, so that CD4 T lymphocyte cell levels will continue to decrease (21, 22). This is supported by research on a strong correlation between the occurrence of OC in the number of CD4 T lymphocytes <200 cells / mm3 on arising clinical manifestations, including the number of species and lesion area (23, 24). Consistent results illustrated in this study were a significant correlation between the number of CD4 T lymphocyte cells with the number of species and lesion area (6-8).

**Conclusions**

Participants with low levels of CD4 T lymphocytes allow the number of *Candida* species growing in the oral cavity to be more diverse with lesions found in the oral cavity. The reverse condition is also possible if the CD4 T lymphocyte levels are high then the possibility of the number of *Candida sp.* growing in the oral cavity and lesion area are getting smaller.

**Acknowledgement:** We would like to thank Hastika Saraswati who helped in translating the paper, Putri Intan Primasari, who helped in the research. We would also like to thank Pepy Dwi Endraswari.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** None

**Ethical Approval:** This study was ethically approved by the ethics committee of Dr. Soetomo General Academic Hospital (1125 / KEPK / IV / 2019).

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Epigallocatechingallate (EGCG) Antifungal Properties for Candida Isolates from HIV/AIDS Patients with Oral Candidiasis in Compare with Fluconazole

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Abstract

Background: Oral Candidiasis (OC) still mainly opportunistic infection problem in HIV/AIDS Patients. Due to increasing report of fluconazole resistant as common antifungal drugs nowadays, there have been many studies focusing on natural substances and its antifungal properties. In this study, a form of green tea extract, named Epigallocatechingallate (EGCG) 1,25% were examined for their in vitro antifungal activity against Candida sp in comparison to fluconazole (2 mg/ml) as standard antifungal agents. Objective: To evaluate the antifungal activity of EGCG in compare with fluconazole against Candida isolates taken from HIV / AIDS patients with OC. Methods: Fourty Candida sp. isolates taken from HIV / AIDS patients with OC in the Outpatient Unit and Inpatient Installation of the Infectious Disease Intermediate Care Unit (UPIPI) Dr. Soetomo, Surabaya. Antifungal activity were evaluated by using microdilution tests. Results: The microdilution test revealed the MIC of EGCG for all Candida sp. was 0.625%, while the MIC of fluconazole was 100% for all Candida sp. There was significant difference (p <0.05) between the MIC values for Candida sp. by fluconazole and EGCG. The MFC values of EGCG was 50%, while value of fluconazole MFC was 100%. Conclusion: Antifungal activity of EGCG with fungistatic and fungicidal effect is better than fluconazole.

Keywords: Antifungal activity, Candida albicans, Candida non-albicans, EGCG, Fluconazole, HIV/AIDS, Oral candidiasis

Introduction

Oral candidiasis (OC) develops in 80%–95% of the patients with Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS). It is described as an opportunistic infection, often involved in the alteration of oral microflora, systemic diseases and reduced immunity of the host. Opportunistic infectious diseases in HIV/AIDS patients can decrease the quality of life in these patients (1, 2).

Candida albicans is the most prevalent and pathogenic species, but other Candida species, such as C. tropicalis, C. krusei, C. glabrata, C. dubliniensis, C. guilliermondii, C. parapsilosis, C. kefyr, and C. pelliculosa, has become a significant cause of infection in patients with HIV/AIDS (3, 4). Majority of the clinically used antifungals suffer from various drawbacks in terms of toxicity, drug-drug interactions, and lack of fungicidal efficacy, and emergence of resistant strains resulting from frequent usage. For example, fluconazole limited because of the high rate of primary and secondary resistance (5). To identify substances that might be alternatives to traditional medicines, studies were conducted on the antimicrobial activity essential oils for the control of OC (6).
Research on alternative therapies using natural ingredients is currently on the rise. The use of natural extracts to treat Candida is becoming popular, one of which is green tea (Camellia sinensis) extract, which is found to have beneficial effects on health, due to its high antioxidant and immunomodulatory effects. Research conducted by Rahayu and colleagues in 2018 on the immunomodulatory effect of green tea extract in immunocompromised mice with Candida using polyphenols and EGCG. After evaluation, it was found that EGCG 1.25% had an immunomodulating effect against C. albicans infection in immunocompromised mice by increasing the expression of IL-8, IL-17A and HBD-2. It was also found that the minimum inhibitor concentration of green tea extract on the growth of Candida albicans was 12.5% and the minimum bactericidal concentration was 25%. Therefore, administering EGCG can provide an immunomodulating effect against oral candidiasis in immunocompromised patients. Therefore, this study wanted to test the sensitivity of green tea extract and nystatin in oral candidiasis patients with HIV. The use of Epigallocatechingallate (EGCG) is expected to have a better sensitivity than fluconazole.

**Methods**

**Strains and Growth Conditions**

This research used 40 isolates divided in two groups 20 isolates of Candida albicans and 20 isolates Candida non-albicans, were obtained from forty patients Candidiasis oral with HIV/AIDS infection. Candida non-albicans species included in this study consisted of 7 species of Candida krusei, 6 species of Candida glabrata, 2 species of Candida dubliniensis, 2 species of Candida parapsilosis, 1 species of Candida tropicalis, 1 species of Candida norvegensis and 1 species of Candida lypoitica. All of the strains were grown on Sabouraud dextrose Agar under aerobic conditions at 37°C for 24 h before the antifungal assays. The yeast (107 cells/mL) suspensions used in the assays were prepared in sterile phosphate-buffered saline (PBS) at pH 7.2.

**Antifungal Tests**

The minimum inhibitory concentrations (MIC) were obtained using the serial microdilution method in culture of 96-well cell plates based on the Clinical and Laboratory Standards Institute (CLSI). Prepare a 96 well microtiter plate that has been filled with Mueller Hinton Broth (MHB) and fluconazole with 10 levels of concentration using a multilevel dilution technique, which is 100%; 50%; 2.5%; 1.25%; 0.0625%; 0.312%; 0.156%; 0.078%; 0.0039%; and 0.0195%. Then enter the EGCG with the highest concentration of 100% in the first row and then do the multilevel dilution along the Y axis to the lowest concentration of 0.19%. Candida species were inoculated into the plate by leaving row one as a negative control. MIC observations are determined by observing at what concentration in the well begins to clear and there is no sediment that indicates the growth of Candida is inhibited. Minimal Fungicidal Concentration (MFC) is tested by taking 10 µL from the well with a predetermined MIC each well is inoculated on a Petri dish containing Sabouraud dextrose agar and incubated at 37 °C for five days. MFC is defined as the lowest concentration without growth that is seen to be used as the end point for the fungicidal effect.

**Statistical Analysis**

The statistical differences were evaluated using SPSS 17 version. The data for microdilution methods were analyzed by chi square on normal data distribution and MannWhitney on abnormal data distribution in statistic. Statistical significance was determined at \( p < 0.05 \).

**Results**

Fourty isolates taken from HIV / AIDS patients with OC. The male patients were 31 patients (77.5%) and female patients were 9 patients (22.5%). The age of patients from isolates in this study varied between the age group 17-25 years to 56-65 years. The most age range is 20 subjects (50%) in the 26-36 years old group. The isolates most were taken from HIV / AIDS patients with OC who had an absolute CD4 count <100 cells/L for 28 patients (70%).

Table 1 shows the results of microdilution, there is a statistically significant difference with the chi square test method at a concentration of 50% between fluconazole and EGCG \( p <0.05 \). At a concentration of 25% there was no significant difference \( p> 0.05 \) and the rest could not be analyzed because all the results were still visible turbidity. MIC was produced by EGCG statistically through chi square test for Candida albicans species.
at a concentration of 50%, while MIC fluconazole at a concentration of 100%.

Table 2 shows with the results of microdilution, there is a statistically significant difference with the chi square test method at a concentration of 50% between fluconazole and EGCG (p < 0.05). From the concentration of 25% until 0.38% concentration there were no significant differences between fluconazole and EGCG (p > 0.05). The MIC produced by EGCG was statistically through the chi square test for Candida non-albicans species at concentrations of 50% while MIC fluconazole at 100% concentration.

Shapiro Wilk test confirmed that the data were not normally distributed (p < 0.05); therefore, nonparametric statistical tests, the Mann Whitney test, was performed. Based on data from Table 3 shows the results of microdilution method research, the MFC values EGCG statistically through the Mann Whitney test is 50%, with this concentration can kill the Candida albicans and Candida non-albicans. The value of fluconazole MFC was 100%. Mann Whitney test results showed there were significant differences between MFC fluconazole with EGCG on the growth of Candida albicans and Candida non-albicans (p < 0.05).

### Table 1. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against Candida albicans.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Treatment Values</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>EGCG</td>
</tr>
<tr>
<td>Concentration 100</td>
<td>(-)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Concentration 50</td>
<td>(+)</td>
<td>19 (95%)</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Concentration 25</td>
<td>(+)</td>
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</tr>
<tr>
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<td>(-)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Concentration 12.5</td>
<td>(+)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Concentration 6.25</td>
<td>(+)</td>
<td>20 (100%)</td>
</tr>
<tr>
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<tr>
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<td>Concentration 0.38</td>
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<td>20 (100%)</td>
</tr>
<tr>
<td>Concentration 0.19</td>
<td>(+)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

### Table 2. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against Candida non-albicans.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Treatment Values</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>EGCG</td>
</tr>
<tr>
<td>Concentration 100</td>
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<td>20 (100%)</td>
</tr>
<tr>
<td>Concentration 50</td>
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</tr>
<tr>
<td></td>
<td>(-)</td>
<td>9 (45%)</td>
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<td>Concentration 25</td>
<td>(+)</td>
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<tr>
<td></td>
<td>(-)</td>
<td>6 (30%)</td>
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<tr>
<td>Concentration 12.5</td>
<td>(+)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td></td>
<td>(-)</td>
<td>3 (15%)</td>
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</tbody>
</table>
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<table>
<thead>
<tr>
<th>Concentration</th>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>Fluconazole</td>
<td>25.0 ± 44.42</td>
<td>0.002</td>
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<tr>
<td></td>
<td>EGCG</td>
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<td>0.021</td>
</tr>
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Table 3. MFC Fluconazole and EGCG for growth *Candida albicans* and *Candida* non-albicans

<table>
<thead>
<tr>
<th>Spesies</th>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
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<td></td>
</tr>
<tr>
<td><em>C. non-albicans</em></td>
<td>Fluconazole</td>
<td>27.5 ± 44.35</td>
<td></td>
</tr>
<tr>
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**Discussions**

Basic data of this study showing isolates mostly taken from male than female (77.5% and 22.5%). The most age group is productive age groups in the age range of 26-35 years with 20 subjects (50%). In 2017 report of the Directorate General of Disease Control and Environmental Health, Ministry of Health Republic of Indonesia, found HIV/AIDS patients more male than women, and related more common in young adult which makes it more likely to engage in unsafe sexual behavior that is at risk of HIV transmission (8). The domicile of HIV / AIDS patients with OC mostly came from Surabaya (90%). This is because most patients have to seek help from the nearest health center with most patients from within the city. The isolates most were taken from HIV / AIDS patients with OC who had an absolute CD4 count <100 cells / L for 28 patients (70%). This data is supported by a 2015 Indian study by Kumar that showed 71.4% of patients with a CD4 cell count <200 cells / µL obtained by the fungus growth of Candida species from OC lesions (9).

The evaluation of the antifungal activity of EGCG against all strains of Candida isolates, showed MIC values ranging from 50%-100% and MIC values fluconazole were varying. MFC produced by EGCG for Candida sp. through the Mann Whitney test is 50%, in which concentration is able to kill the Candida albicans and Candida non-albicans. The value of fluconazole MFC was 100%, which was higher than EGCG. Previous study also found that EGCG perform better antifungal activity based on its MIC and MFC in compare to azole drugs (ketoconazole and fluconazole). EGCG showed a significant inhibitory effect in the growth of Candida sp especially through its ability to destroy the Candida biofilm and inhibit mature biofilm maintenance on its MIC. In an in vitro studies, it was shown that EGCG, EGC and ECG caused metabolic instability of *C. albicans* cultures even at the physiological polyphenol.
concentrations found in green tea. Of the three catechins, EGCG was found to be the strongest in slowing down the formation and maintenance of Candida biofilms and interfering with the formation of biofilms. EGCG was also found to be able to bind strongly with ergosterol. This activity might result in pores creation on fungal cell membranes which eventually leading to fungal cells death. It was also shown that higher EGCG concentrations inhibited the chymotrypsin-like activity of C. albicans in vivo which suggests that the impaired proteasol activity contributes to the metabolic and cellular structural disorders of this fungus (10, 11).

Another study with murine model of oral candidiasis showed that EGCG increased the neutrophil count and decreased the amount of infected cells by C. albicans. The increasing concentration of EGCG leads to the increase of neutrophil count. This might be due to immunomodulatory effect performed by EGCG itself which also beneficial for oral candidiasis therapy, especially in immunocompromised patient as in HIV/AIDS patient (12).

 EGCG also produced synergistic effect when used together with fluconazole or ketoconazole resulting in higher fungicidal activity. The results in 4 species with EGCG (MFC) alone resulted in a reduction of 95.13%, while in synergistic combination resulted in a decrease of 92.27% for fluconazole and 97.51% for ketoconazole, compared to controls. The MIC value of fluconazole/EGCG or ketoconazole/EGCG decreased 3 to 4-fold in compare to the inhibitory effect of those drugs alone. Another study also found that the mechanisms of EGCG inhibitory effect on C. albicans is obtained via key enzymes in the biosynthesis of purines, pyrimidines and some amino acids, and independent of pH (10, 11).

Conclusions

These results highlight the potential of EGCG as an antifungal drug candidate. Based on the data showed antifungal activity with fungistatic and fungicidal effect better than fluconazole. We acknowledge the need to determinate the active compounds that inhibit germ tube formation and their mechanisms of action. However, if these substance is planned to be used in medicinal purposes, issues of safety and toxicity will need to be addressed in the next research.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: None

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8. Putranti A, Asmarawati TP, Rachman BE, Hadi


**Epigallocathecingallate (EGCG) Antifungal Properties for Candida Isolates from HIV/AIDS Patients with Oral Candidiasis in Compare with Fluconazole**

Dwi Murtiastutik1, Cita Rosita Sigit Prakoswa2, Indah Setyawati Tantular3, Yusuf Wibisono1, Afif Nurul Hidayati4, Sawitri1, Muhammad Yulianto Listiawan4

1Lecture, 2Professor, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital, Surabaya, Indonesia, 3Professor, Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 4Associate Professor, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital, Surabaya, Indonesia

**Abstract**

**Background:** Oral Candidiasis (OC) still mainly opportunistic infection problem in HIV/AIDS Patients. Due to increasing report of fluconazole resistant as common antifungal drugs nowadays, there have been many studies focusing on natural substances and its antifungal properties. In this study, a form of green tea extract, named Epigallocathecingallate (EGCG) 1.25% were examined for their in vitro antifungal activity against Candida sp in comparison to fluconazole (2 mg/ml) as standard antifungal agents.

**Objective:** To evaluate the antifungal activity of EGCG in compare with fluconazole against Candida isolates taken from HIV / AIDS patients with OC.

**Methods:** Forty Candida sp. isolates taken from HIV / AIDS patients with OC in the Outpatient Unit and Inpatient Installation of the Infectious Disease Intermediate Care Unit (UPIPI) Dr. Soetomo, Surabaya. Antifungal activity were evaluated by using microdilution tests.

**Results:** The microdilution test revealed the MIC of EGCG for all Candida sp. was 0.625%, while the MIC of fluconazole was 100% for all Candida sp. There was significant difference (p <0.05) between the MIC values for Candida sp. by fluconazole and EGCG. The MFC values of EGCG was 50%, while value of fluconazole MFC was 100%.

**Conclusion:** Antifungal activity of EGCG with fungistatic and fungicidal effect is better than fluconazole.

**Keywords:** Antifungal activity, Candida albicans, Candida non-albicans, EGCG, Fluconazole, HIV/AIDS, Oral candidiasis

**Introduction**

Oral candidiasis (OC) develops in 80%-95% of the patients with Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS). It is described as an opportunistic infection, often involved in the alteration of oral microflora, systemic diseases and reduced immunity of the host. Opportunistic infectious diseases in HIV/AIDS patients can decrease the quality of life in these patients (1,2).

Candida albicans is the most prevalent and pathogenic species, but other Candida species, such as C. tropicalis, C. krusei, C. glabrata, C. dubliniensis, C. guilliermondii, C. parapsilosis, C. kefyr, and C. pelliculosa, has become a significant cause of infection in patients with HIV/AIDS (3,4). Majority of the clinically used antifungals suffer from various drawbacks in terms of toxicity, drug-drug interactions, and lack of fungicidal efficacy, and emergence of resistant strains resulting from frequent usage. For example, fluconazole limited because of the high rate of primary and secondary resistance (5). To identify substances that might be alternatives to traditional medicines, studies were conducted on the antimicrobial activity essential oils for the control of OC (6).

Research on alternative therapies using natural ingredients is currently on the rise. The use of natural extracts to treat Candida is becoming popular, one of which is green tea (Camellia sinensis) extract, which is found to have beneficial effects on health, due to its high its low toxicity with antioxidant and immunomodulatory effects. Research conducted by Rahayu and colleagues in 2018 on the immunomodulatory effect of green tea extract in immunocompromised mice with Candida using polyphenols and EGCG. After evaluation, it was found that EGCG 1.25% had an immunomodulating effect against C. albicans infection in immunocompromised mice by increasing the expression of IL-8, IL-17A and HBD-2. It was also found that the minimum inhibitor concentration of green tea extract on the growth of Candida albicans was 12.5% and the minimum bactericidal concentration was 25%. Therefore, administering EGCG can provide an immunomodulating effect against oral candidiasis in immunocompromised patients (7). Therefore, this study wanted to test the sensitivity of green tea extract and nystatin in oral candidiasis patients with HIV. The use of Epigallocatechingallate (EGCG) is expected to have a better sensitivity than fluconazole.

**Methods**

**Strains and Growth Conditions**

This research used 40 isolates divided in two groups 20 isolates of Candida albicans and 20 isolates Candida non-albicans, were obtained from forty patients Candidiasis oral with HIV/AIDS infection. Candida non-albicans species included in this study consisted of 7 species of Candida krusei, 6 species of Candida glabrata, 2 species of Candida dubliniensis, 2 species of Candida parapsilosis, 1 species of Candida tropicalis, 1 species of Candida norvegensis and 1 species of Candida lypotica. All of the strains were grown on Sabouraud dextrose Agar under aerobic conditions at 37°C for 24 h before the antifungal assays. The yeast (107 cells/mL) suspensions used in the assays were prepared in sterile phosphate-buffered saline (PBS) at pH 7.2.
Antifungal Tests

The minimum inhibitory concentrations (MIC) were obtained using the serial microdilution method in culture of 96-well cell plates based on the Clinical and Laboratory Standards Institute (CLSI). Prepare a 96 well microtiter plate that has been filled with Mueller Hinton Broth (MHB) and fluconazole with 10 levels of concentration using a multilevel dilution technique, which is 100%; 50%; 2.5%; 1.25%; 0.625%; 0.312%; 0.156%; 0.078%; 0.039%; and 0.0195%. Then enter the EGCG with the highest concentration of 100% in the first row and then do the multilevel dilution along the Y axis to the lowest concentration of 0.19%. *Candida* species were inoculated into the plate by leaving row one as a negative control. MIC observations are determined by observing at what concentration in the well begins to clear and there is no sediment that indicates the growth of *Candida* is inhibited. Minimal Fungicidal Concentration (MFC) is tested by taking 10 µL from the well with a predetermined MIC each well is inoculated on a Petri dish containing Sabouraud dextrose agar and incubated at 37 °C for five days. MFC is defined as the lowest concentration without growth that is seen to be used as the end point for the fungicidal effect.

Statistical Analysis

The statistical differences were evaluated using SPSS 17 version. The data for the microdilution methods were analyzed by *Chi square* on normal data distribution and *Mann Whitney* on abnormal data distribution in statistic. Statistical significance was determined at $p < 0.05$.

Results

Forty isolates taken from HIV / AIDS patients with OC. The male patients were 31 patients (77.5%) and female patients were 9 patients (22.5%). The age of patients from isolates in this study varied between the age group 17-25 years to 56-65 years. The age range is 20 subjects (50%) in the 26-36 years old group. The isolates most were taken from HIV / AIDS patients with OC who had an absolute CD4 count <100 cells/L for 28 patients (70%).

Table 1 shows the results of microdilution, there is a statistically significant difference with the chi square test method at a concentration of 50% between fluconazole and EGCG ($p <0.05$). At a concentration of 25% there was no significant difference ($p >0.05$ and the rest could not be analyzed because all the results were still visible turbidity. MIC was produced by EGCG statistically through *chi square* test for *Candida albicans* species at a concentration of 50%, while MIC fluconazole at a concentration of 100%.

Table 2 shows with the results of microdilution, there is a statistically significant difference with the chi square test method at a concentration of 50% between fluconazole and EGCG ($p <0.05$). From the concentration of 25% until 0.38% concentration there were no significant differences between fluconazole and EGCG ($p >0.05$). The MIC produced by EGCG was statistically through the chi square test for *Candida non-albicans* species at concentrations of 50% while MIC fluconazole at 100% concentration.

Shapiro Wilk test confirmed that the data were not normally distributed ($p <0.05$); therefore, nonparametric statistical tests, the Mann Whitney test, was performed. Based on data from Table 3 shows the results of microdilution method research, the MFC values EGCG statistically through the Mann Whitney test is 50%, with this concentration can kill the *Candida albicans* and *Candida non-albicans*. The value of fluconazole MFC was 100%. Mann Whitney test results showed there were significant differences between MFC fluconazole with EGCG on the growth of *Candida albicans* and *Candida non-albicans* ($p <0.05$).

### Table 1. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against *Candida albicans*.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Treatment</th>
<th>Values</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>EGCG</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>(-)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>50</td>
<td>(+)</td>
<td>19 (95%)</td>
<td>0 (0%)</td>
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<td>25</td>
<td>(-)</td>
<td>20 (100%)</td>
<td>19 (95%)</td>
</tr>
<tr>
<td>12.5</td>
<td>(+)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>6.25</td>
<td>(+)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>3.125</td>
<td>(+)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
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<tr>
<td>1.56</td>
<td>(+)</td>
<td>20 (100%)</td>
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<tr>
<td>0.78</td>
<td>(+)</td>
<td>20 (100%)</td>
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<td>0.38</td>
<td>(+)</td>
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<td>20 (100%)</td>
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<tr>
<td>0.19</td>
<td>(+)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

### Table 2. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against *Candida non-albicans*.

<table>
<thead>
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<th>Concentration (%)</th>
<th>Treatment</th>
<th>Values</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>EGCG</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>(-)</td>
<td>20 (100%)</td>
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</tr>
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<td>50</td>
<td>(+)</td>
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<td>(-)</td>
<td>14 (70%)</td>
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<tr>
<td>12.5</td>
<td>(+)</td>
<td>17 (85%)</td>
<td>20 (100%)</td>
</tr>
<tr>
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<td>(+)</td>
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</tr>
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<table>
<thead>
<tr>
<th>Concentration</th>
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<th>20 (100%)</th>
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<tbody>
<tr>
<td>(+)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>0.000</td>
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</table>

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<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
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<tr>
<td>C. albicans</td>
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Conclusions: These results highlight the potential of EGCG as an antifungal drug candidate. Based on the data showed antifungal activity with fungistatic and fungicial effect better than fluconazole. We acknowledge the need to determine the active compounds that inhibit germ tube formation and their mechanisms of action. However, if these substance is planned to be used in medicinal purposes, issues of safety and toxicity will need to be addressed in the next research.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: None

References

The Significance of CD4 to the Number of Grown Candida Colonies in Oral Candidiasis Patients with HIV / AIDS

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Abstract

Introduction: Oral candidiasis is a common symptom in HIV/AIDS patients and in some cases have low CD4 levels. Objectives: Analyzing the correlation of CD4 counts with Candida colonization in oral candidiasis patients with HIV/AIDS. Methods: The research procedure consisted of identifying the type and number of Candida species grown in the participant’s oral cavity, and calculating the CD4 count. The statistical tests used Chi Square, Fisher exact, Spearman correlation test, or Anova test (95% CI; p <0.05). Results: Candida colonization found in group I was 1 – 10 (71.05%), group II was 11-100 (60.53%), and group III was >100 (50.00%; p <0.001). The patient’s lesion area in group I was 3-4 (47.37%), group II was 3-4 (65.79%), and group III was 1-2 (94.74%; p <0.001). Most patients had acute pseudomembranous type, consisting of 81.58% (group I), 92.10% (group II), and 97.37% (group III; p <0.001). Conclusion: This study found a significant correlation between CD4 counts and Candida colonization in oral candidiasis patients with HIV/AIDS. The lower the CD4 counts, the higher the number of Candida colonization in the oral cavity.

Keywords: CD4 count, HIV/AIDS, candida species, oral candidiasis

Introduction

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) infections are still major problems throughout the world and most of the clinical manifestations are oral candidiasis. Candida can spread widely, directly from the oral cavity throughout the body depending on the target organ experiencing immune deficiency. In general, HIV/AIDS patients likely have immune deficiency, and cause individual vulnerability to be detected. CD4 counts in peripheral blood are important when evaluating immune status.1, 2

Common oral candidiasis is estimatedly found in 4.2% of HIV patients and 11.6% of AIDS patients. Some literature stated that CD4 counts <200 per mm³ are found in HIV/AIDS patients.3 The pathogenesis of HIV infection is largely due to a decrease in the number of T-cells (certain types of lymphocytes) that contain CD4 cell surface receptors. The immune status of HIV/AIDS patients can be assessed by measuring the absolute number (per mm³) or percentage of CD4 cells. This is considered a way to assess the severity of immunodeficiency associated with HIV/AIDS. The decreasing number of T cells (CD4) progressively marks the prognosis of HIV/AIDS decreases and increases the chance of other infections. HIV will infect and eliminate Th17 cells, resulting in a reduction in IL-17 secretion which then results in a reduction in b-defensin induction, a downregulation of CXC chemokine expression, a decrease in the production of antifungal cytokines, and the result is a shape transition. Candida albicans forms a hyphae which is pathogenic. This causes Candida, which was initially commensal to turn pathogenic and

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causes a fungal infection in the oral cavity mucosa, so it is called oral candidiasis.4,5

Dr. Soetomo General Hospital, Surabaya, Indonesia, is the highest referral hospital in East Java, Indonesia. The number of HIV/AIDS patients has increased every year. In 2017, 1891 patients were reported for treatment, 1997 patients in 2018, and 1671 patients in September 2019. The number of oral candidiasis patients with HIV/AIDS in 2017 was 30%, 32% in 2018, and 33% by September 2019. Based on the description above, researchers were interested in conducting research aimed at analyzing the correlation of CD4 counts with Candida colonization in oral candidiasis patients with HIV/AIDS.

**Methods and Material**

**Participants**

Participants in this study were HIV/AIDS patients at Dr. Soetomo General Hospital Surabaya, Indonesia. Participant inclusion criteria included patients diagnosed with HIV/AIDS using a rapid test/HIV 3 6,7, having a positive candidiasis diagnosis using 10-20% KOH 8,9, and aged>18 years. Participant exclusion criteria included subjects taking antifungal drugs within 2 weeks before the study and no colonies found on culture examination. Participants in this study first filled out the consent form, in which the patient had received an explanation regarding the benefits and objectives of the study.

**Design**

The study was conducted at Dr. Soetomo General Hospital, Surabaya, Indonesia from May 2019 to September 2019. This research was a cross-sectional study with consecutive admission sampling method. The number of participants was 114 participants, which were divided into 3 groups (each group = 38; Figure 1). We have conducted an ethics test at the ethics committee Dr. Soetomo General Hospital (1125 / KEPK / IV / 2019). The research procedure consisted of identifying the type and number of candida fungi in the participant’s oral cavity and calculating the participant’s CD4 count. The patient will take anamnesis, physical examination, laboratory examination, namely KOH and culture with material from the patient’s mouth rinse water, and blood as much as 3 cc for CD4 examination.

**Figure 1. Flowchart Diagram of Participant Candida Spesies Measurement**

Before checking the type and amount of Candida colonization, patients were told not to do oral hygiene. Participants gargled using 25 ml of sterile water, then the water was put into a sterile bottle. Water from the mouth rinse was immediately sent to Dr. Soetomo General Hospital, Surabaya, Indonesia. The water was shaken slowly, then taken ±5-10 ml and put in 15 ml sterile centrifuge tubes and rotated 2,500 revolutios per minute (rpm) for 5 minutes. Eliminate the supernatant and took the pellet using a sterile pipette, placed it in a glass object and added 10-20% KOH. Candida positive was planted on Sabouraud dextrose agar (SDA) at 37°C for 48 hours.10 The SDA media used was CHROMagar Candida (CHROMagar Candida, France). Candida specimens were also carbohydrate tested to identify Candida species.11 Candida specimens that grew later were counted by specialists in the Department of Microbiology, Dr. Soetomo General Hospital, Surabaya,
Indonesia in a colony forming unit (CFU).

**CD4 Measurement**

Patient’s venous blood samples were taken, then put into K3EDTA-countaining tubes, stored at room temperature, and followed by immunophenotyping for 6 hours. CD4 count calculations used flow cytometry (BD FACSCounttm System; San Jose, CA). Participants were divided into three groups: group I (participant with a CD4 level of \(\leq 100/\mu L\)), group II (participant with a CD4 level of 101 - 200/\(\mu L\)), and group III (participant with a CD4 level of > 200/\(\mu L\)).

**Lesion Area**

The area of participant’s candidiasis oral lesions was assessed using a score of 0 = no lesions, 1 = partial lesions, and 2 = all/all lesions. The locations assessed included the tongue, mucous membrane, and palate with a minimum score of 1 and a maximum score of 6. Calculation of the area of the lesion by adding up the three locations of the assessed lesion.

**Statistical Analysis**

The results of the study were presented in the form of mean ± standard deviation (SD) or median (minimum - maximum) and percentage (%). Static analysis used IBM SPSS Statistic software version 23.0 (IBM Corp., Armonk, NY, USA). Analysis of measurement data used Chi Square test, Fisher exact test, Spearman correlation test, or Anova test with 95% CI and significance level of p <0.05.

**Results**

**Characteristics of Participant**

There were 114 patients who were divided into 3 groups based on CD4 count, with each group consisting of 38 participants. The average age of participants in group I was 43.15 ± 3.67 years, group II was 40.02 ± 10.23 years, and group III was 51.02 ± 3.28 years (p = 0.812). They were divided into several age groups, where most participants were in the age range of 36-45 years (37 participants; 32.46%), followed by the age group of 46 - 55 years (28 participants; 24.56%), and the age group of 26 - 35 years (26 participants; 21.05%). Most participants attended senior high school (55 participants; 48.24%), consisting of 42.10% in group I, 52.63% in group II, and 50.00% in group III (p = 0.015). Most participants were Madurese (60 participants; 52.63%) and followed by Javanese (41 participants; 35.96%). Most of the participants in group I were Madurese (63.16%) and most in groups II and III are Madurese too (47.37%; p = 0.002). Most of the participants were private employees (67 participants; 58.77%), consisting of 52.63% in group I, 76.31% group II, and 47.37% in group III (p = 0.025; table 1).

**Table 1. Demographic Characteristics of Participant**

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<td>II (n=38)</td>
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<tr>
<td>Age (mean ± SD)</td>
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<td>40.02 ± 10.23</td>
</tr>
<tr>
<td>Education (%)</td>
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<td>4 (10.52)</td>
</tr>
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<td>9 (23.68)</td>
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<tr>
<td>Senior high school</td>
<td>16 (42.10)</td>
<td>20 (52.63)</td>
</tr>
<tr>
<td>Undergraduate/Diploma</td>
<td>3 (7.89)</td>
<td>5 (13.16)</td>
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</table>
Patient’s Clinical Description

Most participants complained about the appearance of white patches on the oral cavity (100 participants; 87.72%), followed by participants who complained of red patches and sores on the oral cavity and/or the corners of the lips (7 participants; 6.14%), participants with complained of white and red patches on the cavity mouth (4 participants; 3.51%), and patients with white patches and sores in the oral cavity and/or the corners of the lips (3 participants; 2.63%). Most participants had no pain complaint (64 patients; 56.14%), while the rest (50 patients) complained of pain with an average pain scale of 4.13 ± 0.62. The pain included swallowing pain or accompanied by a burning sensation in the throat. Most patients had lesion location on the tongue (54 participants; 47.37%), followed by lesions on the tongue and mucous membrane (38 participants; 33.33%), and on the tongue, mucous membrane, and lips (11 participants; 9.65%), palate (6 participants; 5.26%), tongue and palate (2 participants; 1.75%), tongue, mucous membrane, and palate (2 participants; 1.75%), and mucous membrane (1 participant; 0.88%). The patient’s physical condition can
Candida Species

Culture results from 114 participants found 149 isolates, where 1 participant was able to produce >1 isolate or >1 species of Candida species. Most of the Candida species grew were Candida albicans as much as 98 species (65.77%), while the rest were 51 non-albicans species (34.23%), including Candida glabrata, Candida tropicalis, Candida krusei, Candida dublinensis, Candida lypolitica and Candida parapsilosis (Figure 3).

Correlation between CD4 Count and Candida Species Colonization

Most participants in group I had >100 Candida species colonization (71.05%), while most patients in group II had the colonization of Candida species of 11-100 (60.53%). On the other hand, the number of Candida species colonization of 1 - 10 and 11 – 100 found in group III were similar (50.00% each; p <0.001). Most participants in group I and II had lesion area in the range of 3 – 4. Meanwhile, most patients in group III had lesion area in the range of 1 – 2 (p <0.001). Most participants in all groups had oral candidiasis of acute pseudomembrane type as much as 81.58% (group I), 92.10% (group II) and 97.37% (group III) (p <0.001).

Discussion

Candida albicans is the most commonly found species in isolates, but non-albican species can also develop into pathogens with various susceptibility to antifungal agents. Various studies have shown that Candida species are found in 61-100% of people infected with HIV/AIDS with oral candidiasis. Candida albicans is one of the most common fungi found in HIV/AIDS patients. CD4 count is a sign of immunosuppression to determine the prognosis of HIV/AIDS. The results of this study showed a significant correlation between CD4 counts and Candida colonization. The lower the CD4 cell count, the more Candida colonization. The condition showed that the decreasing immunity of HIV/AIDS patients indicates normal amount of Candida species, which is the normal flora of the body that can become parasitic colonization to the body.

Factors affecting oral Candida colonization in patients with HIV/AIDS and low CD4 counts are important contributors to increasing the likelihood of
oral Candida colonization. A CD4 cell count <200 cells/mm$^3$ is considered a predisposing factor for oral candidiasis. Positive HIV/AIDS patients without antiretrovirals have a high risk of decreasing CD4 counts. Candida species, especially Candida albicans, normally exists in the oral cavity, but when a person experiences decreasing CD4 counts, the colonization becomes uncontrolled.$^{14,15}$

This study showed that correlation of CD4 with lesion area has a significant relationship, in which the lower the colony, the greater the lesion area. Oral candidiasis is probably the earliest prognosticator of HIV infection. The degree of immune suppression affects the risk, severity and anatomic location of the disease. Oral candidiasis is very common when CD4 counts are less than 300/mm$^3$ and esophagitis counts are less than <100/mm$^3$.4,14,15

White patches in the oral cavity mostly found in pseudomembranous oral candidiasis and plaque hyperplasia are common types of oral candidiasis. Participants with complaints of swallowing pain and burning sensation in the throat are clinical signs of lesions attacking the pharynx and esophagus. Recurrence in oral candidiasis can occur in individuals who have low immune status and/or do not receive appropriate and adequate antifungal therapy.$^8$

Candida infections in immunocompromised patients are often severe, progressively fast, and difficult to treat, and such patients have a definite risk of developing oral candidiasis, which is an important oral manifestation in patients with HIV/AIDS. Age and sex do not have a significant significance in this Candida infection. Individual immunological status has a definite impact on the severity of the disease as reflected by a high colony relationship with a low CD4 count. Given the changing patterns of Candida species, isolation and identification are very important and can help in a better treatment strategy, and thus, get good control of the disease.$^4$

In addition, oropharyngeal candidiasis is considered to be an HIV/AIDS-defining condition where oral clinical features of candidiasis and oropharyngeal candidiasis consisting of white plaque, mouth sores and swelling pain can also be observed. Although Candida albicans is the most species isolated from oral opportunistic infections, an increase in the frequency of infections in the oral cavity caused by non-albicans species, such as Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei and Candida dubliniensis, have been observed in the last decade.$^7,16$

The main target of HIV virus is a subset of lymphocytes derived from the thymus, the helper/inducer cell. The surface of this cell has a glycoprotein molecule called CD4, which is known to bind to the HIV virus glycoprotein envelope. CD4 damage in lymphocytes is one of the causes of the occurrence of immunosuppressive effects by viruses. At present, it has been found that CD4 also exists in other cells, although in lower densities, such as monocytes and macrophages as well as those in tissues such as Langerhans cells in the skin and dendritic cells in the blood and lymph nodes. These cells are also cells that have an important role to start the immune response so that this function is also disrupted by the presence of bonds with the HIV virus.$^2,4,5$

CD4 or similar molecules are also detected in the brain, although it is not clear which cells express the CD4. HIV that has entered into CD4 lymphocyte cells will carry out multiplication by hitching a ride in the process of growth of its host cell. In CD4 lymphocyte cells, HIV replicates and damages these cells, and when they are mature new viruses emerge and then enter other CD4 lymphocyte cells, multiplying and subsequently damaging these cells. A low CD4 T lymphocyte count is said to be the biggest risk factor for current oral candidiasis. An increased risk of oral candidiasis is inversely proportional to the number of CD4 T lymphocytes. The decreasing CD4 T lymphocytes to under 200 cells/μL is a significant risk factor for a more growing colonies in the oral cavity. There is a significant relationship between low CD4 cell count (<200 cells/ml) and oral candidiasis due to immune suppression caused by HIV infection.$^{17,18}$

**Conclusion**

Oral candidiasis patients with HIV/AIDS who have low CD4 levels tend to have a large Candida colonization in the oral cavity, and lesions in the oral cavity are expanding. Patients who are not treated immediately will likely have a broader oral candidiasis, higher number of colonies, wider area of lesion, and lower CD4 T lymphocyte level. This condition represents
a circle that worsens each other’s condition.

**Acknowledgement:** We would like to thank Hastika Saraswati, MD for helping translating this manuscript. We also thank Bernadya Yogatri Anjuwita Saputri, MD, who helped collecting data and Alvin Arifin Saiboo, MD for helping submitting this manuscript. In addition, we thank Pepy Dwi Endraswari, MD for assisting in implementing the culture of Candida species in the Department of Microbiology Dr. Soetomo General Hospital, Surabaya, Indonesia.

**Conflict of Interest:** The authors declare that there is no conflict of interest

**Source of Funding:** Self

**Ethical Clearance:** Taken From Ethics Committee in dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1125 / KEPK / IV / 2019).

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Impact of Bone Marrow Mesenchymal Stem Cells on Different Body Organs of Albino Rats Exposed To Cadmium Chloride Toxicity: A Systematic Review

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Abstract

Background and Objective: Cadmium chloride (Cd cl$_2$) is a highly toxic heavy metal that causes severe degenerative effects to different body organs. Bone marrow mesenchymal stem cells (BMMSCs) may have a protective effect upon CdCl$_2$ induced structural and functional changes in the rat’s organs. This systematic review aimed to test the regenerative effects of bone marrow‐derived mesenchymal stem cells on different body organs of albino rats exposed to cadmium chloride toxicity.

Methods: Experimental animal studies were identified by the following electronic databases: PubMed, Scopus, Ovid Medline, KoreaMed, Google Scholar, Latin American and Caribbean Health Sciences Literature (LILACS) and Central Cochrane Library. The literature search was done from January 2010 to 2020.

Conclusion: From the selected articles, using stem cell therapy enhances the regeneration process in CdCl$_2$ toxicity of different body organs.

Keywords: Cadmium chloride; bone marrow stem cells; rats; systematic review.

Introduction

Civilization advances and increased environmental pollution exaggerate the effects of different xenobiotics as heavy metals on the function of the living organism [1-3]. Heavy metals are defined as trace naturally occurring metallic elements that found throughout the crust of the earth and have high density when compared to water [4]. Human exposure to these metals has been increased dramatically due to their excessive use in industry, agriculture and technological applications [5]. Cadmium is one of the most abundant and toxic heavy metals. It is the 17$^{th}$ most toxic metal and its concentration in earth crust reach about 0.1 mg/kg. It accumulates in body throughout the life. Human exposed to Cd by inhalation and ingestion from plants leading to acute and chronic intoxications. Cd remains in soils and sediments for many years. Plants take up this metal which gets accumulated in them and concentrates along the food chain, reaching the human body [6, 7]. Once absorbed, Cd is rapidly cleared from the blood and concentrates in various tissues. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis [8]. Mesenchymal stem cells are excellent option for cell therapy because they are easily accessible, cells can expand to clinical scales in a relatively short time and...
can be preserved with minimal loss of potency [9]. The regenerative potential of MSCs has been widely studied as it includes in treating tissue lesions caused by ionizing radiation and clinical radiotherapy. Animal studies and early clinical experiences suggested a role for MSCs in the regeneration of these tissue injuries by differentiating into functional parenchymal cells and creating a nurturing microenvironment for other cells [10]. According to PICO, the research question for this systematic review was that ‘dose BMSCs reduce cadmium toxicity in rat’s different body organs. The population was rats; the intervention was use of stem cells to treat cadmium induced toxicity; the comparator was control positive groups that receive cadmium without stem cell treatment; the outcome was the therapeutic effects of stem cells on toxicity induced by Cd in different body organs.

Methods

Protocol development and eligibility criteria: The question was formulated using the PICO format for the research question construction. The methodology of this SR was designed on the basis of the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [11].

Inclusion criteria: All the studies involved in this SR should have the following criteria: (1) Experimental animals exposed to Cd were only rats, (2) The toxicity was induced by cadmium chloride only, (3) Treatment option was BMSCs, (4) Affected structures were different body organs, (5) All included studies must contain control group that receive cadmium chloride with no treatment, (6) The main evaluation is based on the histopathological and/or laboratory evaluation tests and (7) The outcomes were degenerative changes of Cd toxicity in different body organs and regenerative effects of BMSCs.

Exclusion criteria were (1) Animals other than rats, (2) Therapeutic modalities other than BMSCs, (3) Toxicity induced by heavy metals other than Cd toxicity, (4) Studies with no control group, (5) Reviews, (6) Book and book chapter, (7) Case reports, (8) Clinical studies, (9) Studies on human and (10) In vitro studies.

Information sources and search strategy: The PROSPERO and the Cochrane Database of SRs were searched in 10 August 2020 and no existing reviews were found dealing with the effect of BMSCs on Cd-induced toxicity in different body organs. To obtain eligible studies related to our criteria, comprehensive search of peer reviewed literature, published up to 2020, was performed of the electronic databases PubMed, Scopus, Ovid Medline, koreaMed, Google Scholar, Latin American and Caribbean Health Sciences Literature (LILACS) and Central Cochrane Library. The literature search was done from 2010 to 2020. The population was rats; the intervention was use of stem cells to treat cadmium induced toxicity; the comparator was control positive groups that receive cadmium without stem cell treatment; the outcome was the therapeutic effects of stem cells on toxicity induced by Cd in different body organs. As mentioned before, the research question for this systematic review was that ‘dose BMSCs reduce cadmium toxicity in rat’s different body organs. All searches were performed independently by two authors (EH and ME) to identify published articles related to the focused question.

Searching keywords: Search word/term was performed as following: stem cells and cadmium toxicity or bone marrow stem cells and cadmium chloride and rats or stem cells and cadmium and salivary glands and rats or “cadmium chloride” toxicity and bone marrow stem cells in rats.

Study selection and data collection: Two authors (EH&MM) screened all the titles and abstracts, unrelated studies at this stage were excluded. All the potentially related studies were evaluated independently and the relevant studies were extracted by each author. Disagreements between the two authors about study inclusion or exclusion criteria were solved by third author (MEH). The data of interest from the selected studies was tabulated and the following data were obtained: authors and their country, journal (publication year), number of study groups, number of rats used in the study, gender and weight, type of stem cells and their concentration, duration of the study and outcomes. Fields for which information not found in a publication were entered as “unknown.

Assessment of risk of bias (ROB) in included studies: To assess the quality of the articles, two authors (EH&MEG) independently used the SYRCLE’s risk
of bias (RoB) tool [17]. They assessed selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding of the operators), detection bias (random outcome assessment, blinding of outcome assessor), attrition bias (incomplete outcome data, selective outcome reporting) and other sources of bias. Each item was assessed to be low, high, or unclear RoB. “Unclear” means either lack of information or uncertainty over the possibility of bias. Table 1: Any disagreements in the assessment were also resolved by discussion with a third author (MA).

**Statistical analysis:** Degree of chance – adjusted agreement (kappa coefficient value) was used to determine the inter-reviewer reliability.

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</table>

**Results**

The total articles identified from the search through PubMed, Scopus, Ovid Medline, koreamed, Google Scholar, LILACS and Central Cochrane Library were 1967 articles. After filtering, 1259 were excluded, in addition to 43 duplicates were removed. After the eligibility criteria were applied on the remaining 665 articles, five unduplicated studies were included in this review. A flow chart for the selection process is presented in figure1

**Study characteristics:** According to the publication date, the included five studies were divided into one performed in Saudi Arabia at 2015 [16], one in China at 2017 [14], two in Egypt at 2018 [12, 13, 15]. All studies used cadmium chloride to induce toxicity with different concentrations ranging from 0.4-5 mg/kg body weight. One study induce cadmium toxicity by intraperiotonial (IP) injection of 0.4mg/kg body weight five times per week for five weeks [14], two studies used single IP dose of 2 mg/kg body weight [12, 13], one study inject cadmium IP with the dose of 1 ml/kg saline (solvent of Cd) for 3 days 2020 [15] and the remaining study used Cd orally with the dose of 5 mg/kg body wt/day) for 30 days [16]. All studies concentrated on testis as an organ of interest [13-16] except one study had taken liver, kidney and testis as organs for the study [12]. According to the treatment used, BMMSCs used in all studies with concentration ranged from $1 \times 10^6$ in three studies [12,15,16], $1 \times 10^7$ cells in one study [14] and $2 \times 10^6$ in one study [13]. Four
studies injected BMMSCs intravenously (via retro-orbital, penile vein, portal vein and via tail vein)\(^{12,13,14,15}\) and one study via intratesticular injection\(^{16}\).

**Results of risk of bias within the study:** The overall RoB for the included studies was low risk in one study\(^{14}\) and high risk in four studies\(^{12,13,15,16}\) due to the lack of random allocation concealment of the rats in the study groups, unmentioned blindness of investigator and assessor in most of the studies, in addition to unmentioned information about random housing of animals, if there have the same housing conditions or not. In addition, the reporting of the outcome for the most of studies was not mentioned, if there were any damaged or missing samples or not.

**Study of low risk of bias:** Wang et al\(^{14}\) used CdCl\(_2\) to induce damage in testis of rats with dose of 0.4 mg/kg body weight by intraperitoneal injection 5 times per week for five weeks. Five weeks following Cd exposure; 5×10\(^6\) BMMSCs were transplanted into rats by retro-orbital injections on two consecutive days (1×10\(^7\) BMSCs in total) and sacrificing 2 weeks after cell transplantation. H&E stain showed that testes appeared to have varying degrees of injury as atrophy, endothelial cell swelling and reduced layers of mature cells. The apoptosis rate markedly decreased, body weights were higher in stem cell group than Cd group and greater Cd accumulation was detected when compared with the control group. Histologically, there was a significant improvement in the pathological changes caused by Cd in model group including more cell layers and regularly arranged spermatogenic cells layers. The status of rats improved significantly compared with the model rats.

**Studies of high risk of bias:** Elbaghdady et al\(^{12}\) used CdCl\(_2\) to induce testicular toxicity; each rat received 2 mg/kg IP Cd dissolved in normal saline. Sperms collection, counting and viability were assessed. A lesser weight gain was recorded in Cd-group as compared to the controls. Histologically; necrosis, marked interstitial fibrosis and infiltration of mononuclear inflammatory cells are the main obvious changes. Testis showed an overall atrophy thus; the study supposed that Cd impairs the reproductive capacity caused by the severe testicular damage. BMMSCs used to treat Cd-induced toxicity in testis. Each rat received 2 mg/kg intra-peritoneal Cdcl\(_2\) dissolved in normal saline then the first dose of 1×10\(^6\) stem cells in 0.2 ml DMEM injected intravenously via penile vein. After one week, the second dose of 1×10\(^6\) SCs was given. Results showed that there was an overall reduction in the severity of Cdcl\(_2\)-induced pathological damage after BMMSCs treatment. Higher body weight gain and higher sperm count and sperm were recorded in comparison with the Cd-group. Elbaghdady et al\(^{13}\) also used Cd to induce hepato-renal and testicular toxicity. 2 mg/kg Cdcl\(_2\) dissolved in normal saline was injected IP for each rat. When compared to the control group, the rats treated with Cdcl\(_2\) showed a great decrease in the total number, motility and viability of sperm. There were high levels of abnormal sperm morphology. Lipid peroxidation and oxidative stress were associated with the increase in testicular necrosis and lowered sperm count and viability. Significant abnormalities in all measured biochemical analyses were documented. Rats showed marked decrease in total protein levels and significant increases in serum uric acid and creatinine levels and this may be due to kidney damage caused by CdCl\(_2\) exposure. After Cd treatment, the rats received two successive doses of MSCs separated by one week, each dose of 1×10\(^6\) cells/ rat suspended in 0.2 ml DMEM via intravenous injection into the portal veins. Results showed a statistically significant increase in sperm total count, viability and motility in comparison with the controls. Improvement in all biochemical measures were recorded to the extent that they reached the normal values. The microscopic examination of liver sections of stem cells treated animals showed marked reduction in the hepatic vacuolation, mild leukocytic infiltration and significant decrease in cell degeneration. Abdel Latif et al\(^{15}\) used CdCl\(_2\) to induce toxicity in their study. CdCl\(_2\) was dissolved in normal saline and injected intraperitoneally, each rat received1 mg/ kg for consecutive three days. Rats were received BMMSCs injection once via tail vein, at a dose of 1×10\(^6\) cells/ rat suspended in 0.2 ml PBS after Cd treatment for 3 days and remained for 4 weeks. Sections of the stem cell-treated group showed normal architecture of tubules, minimal fluid exudates and vacuoles. Serum levels of testosterone showed marked increase (137%) while MDA and NO levels were decreased as related to non-treated group. Moreover, there were 154% significant increases in the mean values SOD and 138% significant increments in the mean values of GSH in relation to that of non-treated group. BAX level recorded significant decrease, however Bcl-2 levels
were decreased. Hussein et al\textsuperscript{16} induced toxicity to rats using Cd at the dose of 5 mg/kg body weight dissolved in water daily via oral administration for 30 days then blood samples were collected from retro-orbital plexus and used for detection of sex hormones using ELISA, liver and kidney functions and total antioxidant capacity. Oxidative stress parameters (MDA and NO) contents and antioxidant parameters \{reduced glutathione (GSH), Superoxide dismutase (SOD) activity and CAT activity\} were estimated. Results revealed that there was marked decrease in the body weight after 4 weeks of Cd ingestion with gradual and sustained significant decrease in the weights of left and right testis. There was a significant decrease in serum levels of testosterone and LH and marked elevation in FSH, prolactin serum levels, MDA, NO, CAT, ALT, AST, ALP, creatinin and urea levels in relation to control values, while total protein and albumin were not affected. Decrease in sperm count and sperm motility was recorded. BMMSCs were used to regenerate damaging effects of Cd. 24 hours after the last dose of cdcl\textsubscript{2}, animals were received a single intratesticular injection of rat BMMSCs containing \(1 \times 10^6\) cells then left for 30 days. Results revealed that treatment of cd-exposed rats with stem cells caused body weight gain, reversed or fixed all injuries caused by cdcl\textsubscript{2} and retained to control levels. Significant improvement in the sperm count and motility so, they indicated that BMMSCs may play an important role in recovering the testicular function of adult rats.

\textbf{Discussion}

This systematic review aimed to assess the therapeutic and protective effects of BMMSCs as a treatment option for Cd induced toxicity, so the experimental animal chosen for this review was the albino rats, as they provide an appropriate model to study naturally or experimental occurring cadmium toxicity to different body organs. All the included studies were published in the last 10 years. Heavy metals are metallic elements that have high density compared to water \textsuperscript{18}. There is an increasing ecological and global public health concerns related to environmental contamination by these metals and exposure of human has increased as a result of their frequent use in many industrial, technological and agricultural applications \textsuperscript{5}. Industrial progress has brought human into close contact with several injurious chemicals, including heavy metals such as cadmium, lead and mercury. Cd is a highly toxic metal with widespread exposure to human causing tissue damage that lack effective treatment\textsuperscript{14}. It presents at low levels but human activity as tobacco smoking has greatly increased its level. Exposure to this metal can occurs in the workplace and in the environment as it is utilized in a number of industrial practices and is considered as a contaminant of the environment and dietary products \textsuperscript{19}. Cadmium accumulation occurs mainly in the soft tissues as the liver, kidney and testes \textsuperscript{20, 21}. This metal acts as a catalyst so its toxicity associated with oxidative tissue damage. Cd increases the production of ROS as a result of its inhibitory effects on mitochondrial electron transport \textsuperscript{22}. The present review aimed to investigate the effect of injected BMSCs to decrease the hazardous effects of Cdc\textsubscript{2} which induced changes in some biochemical and histological parameters in different body organs of rats. It was concluded that BMMSCs have the ability to improve and recover the cellular damage induced by Cd exposure in testis, liver and kidney of rats. It was not possible to perform a direct comparison between the selected studies due to different variations between them in number of stem cells implanted, concentration of Cd, route of toxicity induction, period of the experiment and the organs of interest, so quantitative meta-analysis of the data could not be carried out. Irrespective of all of that difference, the majority of the studies demonstrated a statistically significant improvement in the outcome measures between the groups used stem cells as a treatment for Cd induced toxicity. After all of that, the present review has some limitations. Firstly, studies selected should be written in English, but this under represents the studies written by any language other than English. Secondly, the finding of most of the included studies don’t based on follow up periods even short follow up periods as long follow up period will be difficult to be done in animal studies. Thirdly, the toxicity induction in these studies has different concentrations, periods and organs of concern. Also stem cells used of different numbers and routes of administration that may affect the regeneration.

\textbf{Conclusion}

The finding from this systematic review demonstrates that Cd induces great injury to different body organs. Using stem cell therapy enhances and improves the regeneration process in Cd toxicity of
different body organs so they can be used as a therapeutic potential to overcome harmful effects of such toxicity.

**Ethical Clearance**- Taken from faculty of dentistry ethical committee, Mansoura, Egypt

**Source of Funding**- Self.

**Conflict of Interest** - Nil.

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Diagnostic Accuracy of Recto-Sigmoid Index Evaluation on Barium Enema in Hirchsprung’s Disease

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Abstract

Background: Hirschsprung’s disease is a congenital disorder caused by the absence of ganglion cells in the bowel wall, which results in functional obstruction of the aganglionic segment due to failure of relaxation during peristalsis. The recto-sigmoid index on barium enema may constitute in the diagnosis of Hirschsprung’s disease.

Aim: To evaluate the sensitivity, specificity, positive predictive value, and negative predictive value of recto-sigmoid index for the diagnosis of Hirschsprung’s disease in patients who underwent barium enema examination.

Methods: This retrospective study was conducted in Dr Soetomo Hospital. Medical records of patients suspected of Hirscsprung’s disease who underwent both barium enema examination and diagnostic full thickness biopsy were evaluated. Moreover, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of recto-sigmoid index was calculated.

Results: A total of 37 patients were examined; 21 of them were male. The average age was 24.5 (SD 17.34, range 1 month-72 month). Biopsy revealed the absence of ganglion cells in 32 (91.8%) patients. The recto-sigmoid index agreed with the histopathologic diagnosis in 30 (81%) patients. The sensitivity, specificity, PPV, and NPV were 87.5%, 40%, 90.5% and 33% respectively.

Conclusion: Recto-sigmoid index evaluation in barium enema for the diagnosis of Hirscsprung’s disease has poor diagnostic accuracy.

Keywords Hirschsprung’s disease; aganglionic; recto-sigmoid index; barium enema

Background

Hirschsprung’s disease (HD) is a congenital disorder in the form of parasympathetic aganglionic segments in the submucosa or Auerbach’s plexus of the colon, from the internal anal sphincter to the proximal with varying lengths. The clinical symptoms are functional bowel disorders. This disease is caused by the cessation of the craniocaudal migration of the neural crest cells in the distal colon in the fifth week to the twelfth week of pregnancy that functions to form the intestinal nerve system.\textsuperscript{1}

HD occurs in 1 out of 5,000 births and it is the most common cause of neonatal lower gastrointestinal obstruction.\textsuperscript{2} This disease shows a predominance of men over women with a ratio of 4:1. The incidence of HD increases in familial cases to an average of about 6% (ranged between 2% and 18%).\textsuperscript{3}
Specimen examination using full thickness biopsy is the gold standard in enforcing the HD method. This examination must be carried out immediately on fresh specimens, so it is often difficult to carry out due to technical difficulties and a shortage of experts. In addition, this examination is invasive, requiring general anesthesia and sutures at the biopsy site.

The border area between the ganglion and aganglionic segments is called the transition zone, which is usually found in the recto-sigmoid region. This zone can be detected using a radiological examination, namely barium enema. In barium enema examinations, the recto-sigmoid index (RSI), which is the ratio of the widest diameter of the rectum to the widest diameter of the sigmoid colon, is calculated. However, the RSI often gives many false negative results in a certain age group, particularly under 1 month of age.

This research aims to determine the accuracy of RSI in the diagnosis of Hirschsprung’s disease using barium enema by calculating the sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio of RSI.

Methods and Materials

This research is a diagnostic test that applied the cross-sectional method. It was conducted from February 2017 to May 2017 at Dr. Soetomo General Hospital Surabaya. The research samples are infants and children suspected of Hirschsprung’s disease who were treated in the neonatal ward, pediatric ward, and operating room of the hospital and underwent barium enema and histopathological examinations. Those whose medical records contain incomplete patient characteristics were excluded. The data used in this research are secondary data collected from the medical records of inpatients with gastrointestinal conditions in the neonatal and paediatric wards of the hospital from February 2017 to May 2017.

The diagnostic accuracy of RSI was obtained by comparing it with the results of full thickness biopsy with calretinin staining, which was used as the gold standard. The RSI was calculated by a radiologist at Dr Soetomo Hospital. If the RSI is greater than 1, it does not support HD, but if the RSI smaller than 1, it supports HD. Unprepared barium enema examination was carried out using a tool from Canon manufactured in 2000. The accuracy is good if the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio (LH) have a high value.

Meanwhile, full thickness biopsy was performed by a pediatric surgeon at Dr Soetomo Hospital. The result is positive if ganglia and nerve fibers are not found in the submucosa and muscular layers and negative if ganglia and nerve fibers are found in those layers.

Results

36 subjects between the age of 1 and 72 months with medical records containing a biopsy and barium enema evaluation met the inclusion criteria. Based on their sex, 20 subjects were male and 16 were female. 31 subjects were born at term, the mothers of 2 subjects experienced preeclampsia and eclampsia, and malnourishment was found in 21 subjects. The characteristics of the subjects are presented in Table 1.

Table 1 Characteristics of Research Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Quantity</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>44.73</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>55.27</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum: 1 month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum: 72 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean: 24.5 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term</td>
<td>32</td>
<td>88%</td>
</tr>
<tr>
<td>Premature</td>
<td>4</td>
<td>11%</td>
</tr>
<tr>
<td>Nutritional Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-nourished</td>
<td>3</td>
<td>8%</td>
</tr>
<tr>
<td>At risk for malnutrition</td>
<td>21</td>
<td>58%</td>
</tr>
<tr>
<td>Malnourished</td>
<td>5</td>
<td>14%</td>
</tr>
</tbody>
</table>
Table 2 shows the clinical illustration of the research subjects. Abdominal distension, which occurred to 33 research subjects, was the most frequent complaint. It was followed by delayed meconium passage or delayed first defecation that occurred to 32 patients; constipation, which was experienced by 30 patients; and another congenital disorder, which was down syndrome that occurred to 2 patients.

**Table 2 Clinical Illustration**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Quantity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Distension</td>
<td>33</td>
<td>92</td>
</tr>
<tr>
<td>Delayed Meconium Passage</td>
<td>32</td>
<td>90</td>
</tr>
<tr>
<td>Fecal Incontinence after Rectal Examination</td>
<td>28</td>
<td>84</td>
</tr>
<tr>
<td>Constipation</td>
<td>30</td>
<td>88</td>
</tr>
<tr>
<td>Down Syndrome</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

32 (82%) subjects had positive biopsy results. If highlight is found on ganglia with or without nerve fibers, Hirschsprung’s disease is excluded. Conversely, if highlight is not found on ganglia in the microscope slide, it can be inferred that the patient suffers Hirschsprung’s disease. Table 5.3 shows the results of S100 staining on the full thickness biopsy specimen. The biopsy resulted in 32 positive and 3 negative cases.

**Figure 1. Microscopical Imaging of Immunohistochemistry Test Using Calretinin on Full Thickness Biopsy Specimen: Auerbach’s ganglion is not found between the muscularis propria layers (400X magnification).**

The RSI evaluation results of 30 (81%) subjects supported the diagnosis of HD.

**Table 3 Recto-sigmoid Index of Colon in Loop**

<table>
<thead>
<tr>
<th>Result</th>
<th>Quantity</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>30</td>
<td>81</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table 5 presents the comparison of the recto-sigmoid index in barium enema examinations and the full thickness biopsy results.

**Table 4 Comparison of RSI and Biopsy Gold Standard**

<table>
<thead>
<tr>
<th>Method</th>
<th>Biopsy</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>RCI &lt; 1</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>RCI &gt; 1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>5</td>
</tr>
</tbody>
</table>

The diagnostic values of RSI evaluation can be seen in Table 6. The sensitivity and specificity were 87.5% and 40% respectively. The positive predictive value of anti-*H. pylori* IgG in urine was 90% and the negative predictive value was 33%. The negative and positive likelihood ratio of anti-*H. pylori* IgG was 0.32 and 1.45 respectively.

**Table 5 Diagnostic Accuracy of RSI examination in the diagnosis of Hirschsprung’s Disease**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>87.6</td>
<td>1.5-36.4</td>
</tr>
<tr>
<td>Specificity</td>
<td>40</td>
<td>61.6-98.4</td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>90</td>
<td>6.7-93.2</td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>33</td>
<td>29.5-67.4</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>1.45</td>
<td>0.15-5.90</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>0.32</td>
<td>0.78-1.3</td>
</tr>
</tbody>
</table>

**Discussion**

The research subjects were dominantly male, which constituted 55.27% (20 patients), whereas the female patients were 44.73% (16 patients). 32 (88%) patients were born at term and only 4 patients (12%) were born preterm with gestational age under 37 weeks. Abdominal distension was experienced by 33 (91%) patients, delayed meconium passage by 30 (88%) patients, and chronic constipation by 30 (90%) patients. These characteristics are in line with the results of previous research, which revealed that most HD patients are male,8,9,10,11,12,13 were born at term,14,15 as well as experienced abdominal distension (Chirdan, 2000; Nasir et al, 2007), delayed meconium passage,16 and chronic constipation that usually persists despite therapy using laxatives.17

Down syndrome was included in the research subject characteristics because the syndrome is a genetic disorder that often accompanies HD.18 Out of 36
patients, 2 (13%) of them have Down syndrome. This is in accordance with the previous research, which found that the prevalence of Down syndrome in HD patients ranges from 10% to 13%.19,20

In this research, four samples that were examined using full thickness biopsy with calretinin staining showed a positive imaging, indicated with blackish-brown color on the ganglia in the submucosa, muscularis propria, and/or lamina propria with or without nerve fibers. Calretinin reagent makes cells or tissues containing calretinin protein into brown, including tissues of the brain, heart, intestines, etc. The colored cells are not only ganglion and neuron cells, but also the morphology unique to ganglia and fibers that are easily recognized in specimens stained with calretinin.21

A diagnostic test has a high value if the sensitivity and specificity is ≥ 90%.22 The sensitivity, specificity, PPV, and NPV were 87.5%, 40%, 90%, and 42% respectively. Other results of anti-\textit{H. pylori} IgG in urine in other countries can be found in Table 7.

The sensitivity of the RSI in this research was 87.5%, which is considered adequate for the initial screening of a disorder or a disease. In general, previous research that assessed the accuracy of RSI resulted in adequate sensitivity, but some argued that the sensitivity is better as the patient ages because the older the patient, the more visible the transition zone. The research based on age groups conducted by Garcia et al.7 showed that the highest sensitivity value was found in the group of above 1-month old patients. However, transition zones that indicate HD are not always present in HD cases. Rosenfield et al.23 discovered that the transition zone is not found in the radiology imaging in HD-positive cases, RSI shows positive results in 32 of 42 patients (72%), and positive cases are more frequently found in children than neonatal subjects.

This research has low specificity, which was only 40%. It suggests that the RSI in the barium enema examination is not good enough to be used to diagnose HD, which may lead to inaccurate management and unwanted outcome, such as discomfort and disappointment of patients, the need of further tests, and the use of unnecessary resources and additional costs.24

The PPV and NPV in the RSI evaluation were 90% and 33% respectively. It means that the subject has 90% probability to suffer from \textit{H. pylori} infection if the test result is positive. Conversely, the subject has 33% probability to not suffer from the disease if the test result is negative.

Likelihood ratio is also a determining value of a diagnostic test. The higher the LR (positive), the more accurate the test in diagnosing which subject is ill and which is not.22 This research resulted in the LR (positive) value of 1.45. Although it is greater than 1, the LR value is insignificant. LR value is significant if the difference is 10 or higher.22

The evaluation of RSI in barium enema examinations can show false negative and false positive results. False negative results in this research may have been caused by the failure to visualize the transition zone in the barium enema examinations due to various factors, such as the inaccurate procedure during rectal washout and inaccuracy in reading or interpreting the imaging results. In certain cases, the causes can be the unclear visualization of the total aganglionic and long HD segments, as well as transition zones. In fact, transition zones are not found at all. Some research emphasized that the transition zone is more visible as the patient ages. In infants under 1-month old with delayed meconium passage of over 24 hours and several cases, the transition zone does not appear (Garcia et al., 2013).

On the other hand, false positive results in this research may have been caused by other factors that resulted in the imaging of transition zones in the reading of barium enema examination. Some of them are meconium plug, microcolon, and colonic atresia and stenosis.25

**Conclusion**

It can be concluded that the evaluation of RSI in the barium enema examinations had adequate sensitivity, but it had low specificity, so it cannot be used as the sole diagnostic tool in the diagnosis of HD. The high positive predictive value indicates that if the test result is positive, it can be used as the basis of a more specific examination, namely rectal biopsy. Further prospective research involving more subjects of certain age groups is necessary to obtain results with good accuracy.
Conflict of Interest: None declared.

Source of Funding: The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Clearance: Approved by researched ethical committee Dr. Soetomo General Hospital Surabaya No: 1787/KEPK/I/2020.

References


Edible Oils Consumption and the Fatty Acids Profile of the Most Common Consumed Brands in Shiraz, Iran

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Abstract

Aim: This study aimed to measure edible oils consumption, the most preferred brands and their fatty acid profile compared with standard values in Shiraz, Iran.

Methods: In this cross-sectional study 384 households were assessed. Edible oil types (frying, sunflower, corn, canola, rapeseed, mixed, solid, olive, grapeseed, sesame, and animal), brands, volume of consumed, attention to the label, and changes in the volume of oil consumption compared to the last year were measured. Resistance, fatty acid patterns, total trans fatty acid, smoke point, and saturated fatty acids of the three most frequently consumed edible oils were compared to the standard recommended values.

Findings: The mean of households oil consumption was 40.34 ± 32.88 kg per year. Frying (78.6%), sunflower (60.7%), and olive (37.5%) oils were the most common consumed oil. 57.6% of participants read the labels. 28.9% could not understand the labels information. Compare to the last year, 53.8%, 46.8%, and 55.5% of studied households reported decrease in the liquid, solid and frying oils consumption. Fatty acid profile in majority of studied brands were in the standard range. Myristic acid level in the three major brands of solid oil was out of the standard range. The level of C16:1, C18:1, C18:2, C18:3 in the third common olive oil, C14:0 and saturated fatty acid in the third common frying oil were out of the standard range. The resistance level in the third common corn oil, the first common and the third common frying oil was out of the standard range.

Conclusion: Liquid, solid and frying oils consumption among studied population decrease than the last year, which can be helpful to reduce the risk of chronic diseases. The fatty acids profiles of the most common consumed brands were in the standard range.

Key words: Edible oils consumption; Fatty acid profile; Saturated fatty acids

Introduction

Noncommunicable diseases are one of the main public health challenges in the 21st century. Globally, these diseases are the leading cause of death with unacceptably high burden(1). In 2016, 71% of the world’s deaths were related to noncommunicable diseases. 78% of all noncommunicable diseases deaths occurred in low- and middle- income countries(2). Cardiovascular diseases, cancers, chronic respiratory diseases, and diabetes are the major noncommunicable diseases responsible for these deaths(3). Global Noncommunicable Diseases Action Plan focused on reducing risk factors prevalence as the main effort to prevent and control these diseases(4).

Unhealthy diet is among risk factors for noncommunicable diseases. It is known that the dietary factors have an important role in the development and prevention of these diseases(1). Obesity, as one of the main risk factors for many noncommunicable diseases,
is directly related to unhealthy diet and less physical activity\(^5\). Dietary habits with high trans fats, saturated fats, sugar and salt are associated with increased risk of cardiovascular diseases, diabetes, and hypertension\(^6\). Also, it is shown that the risk of cardiovascular disease and diabetes are influenced by oils fatty acid profile. The risk of cardiovascular events increased following dietary content of high saturated fatty acids. And the higher risk of both cardiovascular disease and diabetes was associated with high intake of trans fat\(^7\-\(^8\). So, fatty acid reduction policies were considered. The World Health Organization, in 1994, recommended that less than 4% of the total fat as trans should be found in oil consumed\(^9\).

Edible oil intake, as one of the important sources of fatty acids, plays key roles in human health. Edible oils are used by consumers in the preparation of everyday meals\(^10\). The type of oil consumed can be relate to risk factors associated with noncommunicable diseases. In the human body, low-density lipoprotein can be raised following excessive consumption of saturated fats and cholesterol\(^11\-\(^12\). Some oils with possible high levels of fatty acids like erucic acid or trans isomers of linolenic acid can be harmful for human health\(^13\). In the other hand some fatty acids that cannot be synthesized by human body are essential and need to be solely supplied through the diet\(^14\). Some oils that are unsaturated or contain omega-3 fatty acids or gamma linoleic acid are healthful\(^15\-\(^16\). So, it is important to examine the composition of edible oils in dietary intake across the world.

Iran as a developing country with a population more the 80 million fasses with burden of noncommunicable diseases. The changing dietary patterns is one of attributed factors to the rise in disease burden in Iran as the same as in the world\(^17\). The commonly used variety of cooking oils in Iran are solid and liquid oils, vegetable oils and animal oils\(^18\). The per capita consumption of edible oils among Iranian population has been increased remarkably\(^19\). Evaluation of household’s patterns of the consumption of oils and fats, and brand preference of consumers for edible oils can be useful for policy makers. Little data is available regarding the status of edible oils consumption in Iran. So, the present study was designed to evaluate oils consumption, the most preferred brands in a sample of Iran population. Also, the fatty acid profile and physicochemical characteristics of frequently consumed edible oils were assayed and compared with reference value recommended by the Iranian Standards Organization.

**Materials and Methods**

This cross-sectional study was carried out on different edible oils consumed by urban households in Shiraz, Iran between July to September 2019. Participants included 384 individuals who willing to participate in the study. Those of the participants who did not response half of the questions were excluded. The protocol of the study was approved by the Ethics Review Board of the Shiraz University of Medical Sciences.

The participants were selected using random cluster procedure. Five of eleven municipal zones (as clusters) were randomly selected. Individuals in each area were randomly selected from the malls and supermarkets consumers in a simple random manner based on the population ratio of that area. Selected consumers (preferably the mother of the household) invited to participate in a face-to-face interview by trained interviewer. A questionnaire included questions about sociodemographic characteristics and brand preferences and consumption pattern of edible oils was used for data collection. Sociodemographic data included age, family size, marital status, job, education, family income, and presence of chronic diseases in the family. Data about the participants’ pattern of oil consumption included edible oil types (frying, sunflower, corn, canola, rapeseed, mixed, solid, olive, grapeseed, sesame, and animal), edible oil brand, volume of used edible oils during the last week, consumers’ attention to the oil label, and changes in the volume of oil consumption compared to the last year (as decreased, no change, increased). Eleven brands reported by participants are coded in alphabetical order from A to K.

The three most frequently consumed edible oils, based on participants’ responses, were selected to analyses the oils physicochemical profiles by comparison to reference standards. For each type, three frequently consumed brans were purchased and sampled in coded packages (as A, B and C). The coded samples were sent to a unique laboratory. Resistance to heat, fatty acid patterns, total trans fatty acid, smoke point, and saturated fatty acids of the oils were measured by standard methods.
High-performance liquid chromatography method\(^{(20)}\) was used to measured fatty acids including Lauric acid (C12:0), Myristic acid (C14:0), Palmitic acid (C16:0), Palmitoleic (C16:1), Margaric acid (C17:0), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2), Linolenic acid (C18:3), Arachidonic acid (C20:0), Eicosenoic acid (C20:1), C20:2, Behenic acid (C22:0), Erucic acid (C22:1), C22:2, Lignoceric acid (C24:0), and Elaidic C18:1T. The Rancimat technique was used to measure the oils resistance\(^{(21)}\). Measured values were compared to the standard values recommended by the Iranian Standards Organization and reported in three categories including in standard range, not in standard range and not measured (if the measuring was not necessary).

All statistical analyses were done using SPSS software for Windows (SPSS, Inc., Chicago, IL, USA, version 24). Findings reported as mean ± SD, number (%) or median [IQR] as appropriate. Multivariate logistic regression analysis was used to assessed factors associated with the use of each consumption oils. Odd Ratio with 95% confidence interval (CI) are reported for each factor and the statistical significance of the relationship was inferred from the CI.

### Findings

384 individuals participated in this study. The characteristics of the study participants are presented in table 1. The mean of age was 39.4 years and 90.1% were married. Most of the subjects (69.8%) were housekeeper and 45.6% were overweight or obese. Hypertension (19.8%), hyperlipidemia (16.1%), and type 2 diabetes (15.4%) were the most common comorbidities in the studied participants according to their self-reports.

The mean of households\(^{\square}\) oil consumption was 40.34 ± 32.88 kg per year (110.5 grams per day). Of studied households, 66.4% (255 participants) reported that they reuse the consumed oil. 39.8% (153 participants) reported that they use the oil in low flame heat, 56.1% (198 participants) use the oil in moderate flame heat and 8.6% (33 participants) high use flame heat during oil consumption. 246 participants (64.5%) reported that they keep the oil in low flame heat, 32.3% (124 participants) keep the oil outside the cabinet and 36% (14 participants) reported that they keep the oil inside the refrigerator. Table 2 and 3 presented the oil consumption and brand preference of the studied participants for different types of oils. Households consume more frying (78.6%), sunflower (60.7), and olive (37.5%) oils than the other oils. The annual consumption of oil among households who consume sunflower oil (24 kg), solid oil (20 kg), frying oil (18 kg) and corn oil (18 kg) was more than the other oils. For frying oil, B (41.1%), G (13.9%) and C (12.6%) were the most brand preference of the households. For sunflower oil, B (40.3%), C (23.2%) and A (11.2%); For solid oil, C (44.6%), J (10.9), and E (6.9%) were the most popular brand of the households (Table 2). Also, for olive oil, A (16.7%), Imported edible oils (16.0%), and Home-made oils (11.8%) were the most brand preference of the households (Table 3).

Multivariate logistic regression was used to find factors associated with the type of oil consumption in studied participants (Table 4). Factors significantly associated with the use of cooking oil were age (OR, 1.04), academic education (OR, 3.26), income between 30-50 million Rials (OR, 2.54) and stroke (OR, 3.88). Older participant’s age was significantly associated with lower use of sunflower oil (OR, 0.96). Participants who had a diploma (OR, 0.37) or academic education (OR, 0.27), preferred lower solid oil than those who were under diploma. Higher number of family size (OR, 1.53) and the family presence of hypertension (OR, 2.71) were significantly associated with increasing use of frying oil. Older age (OR, 1.03), having diploma (OR, 2.01), academic education (OR, 2.65), and the family presence of hyperlipidemia (OR, 1.83) were significantly associated with increasing use of other oils.

Of the study participants, 57.6% (n=221) reported to read the labels on the oil container and 28.9% (n=111) reported that they could not understand the labels information. Of 331 participants who consumed liquid oil, 53.8% reported decrease in the oil consumption than the last year. Of 109 subjects who consumed solid oil, 46.8% reported decrease in the oil consumption, and 55.5% of 308 subjects who consumed frying oil reported decrease in the oil consumption than the last year.

Fatty acid patterns and resistance in the major brands of oils according to the Iranian national reference standard are presented in Table 5. Fatty acid patterns and resistance to heat in the three major brands of sunflower oil were in the standard range. Myristic acid level in the
three major brands of solid oil was out of the standard range. The level of C16:1, C18:1, C18:2, C18:3 in the third common olive oil (C) were out of the standard range. Fatty acids including C16:0, C18:0, C18:3, C20:0 in the second common corn oil (B) were out of the standard range. Fatty acids including C18:3 and C20:0 in the first common sesame oil (A) and C18:0, C18:2, C18:3, C20:0 in the third common sesame oil (C) were out of the standard range. C14:0 and saturated fatty acid in the third common frying oil (C) were out of the standard range. Also, the resistance level in the third common (C) corn oil, and the first common (A) and the third common (C) frying oil was out of the standard range.

Table 1: Characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>39.4 ± 10.6†</td>
</tr>
<tr>
<td>Family size</td>
<td>3 [3-4] ††</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>346 (90.1) *</td>
</tr>
<tr>
<td>Single</td>
<td>38 (9.9) *</td>
</tr>
<tr>
<td>Job</td>
<td></td>
</tr>
<tr>
<td>Housekeeper</td>
<td>268 (69.8) *</td>
</tr>
<tr>
<td>Employed</td>
<td>82 (21.4) *</td>
</tr>
<tr>
<td>Self-employment</td>
<td>34 (8.9) *</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>Under diploma</td>
<td>100 (26.0) *</td>
</tr>
<tr>
<td>Diploma</td>
<td>115 (29.9) *</td>
</tr>
<tr>
<td>Academic</td>
<td>169 (44.0) *</td>
</tr>
<tr>
<td>Family income (Rials)</td>
<td></td>
</tr>
<tr>
<td>&lt;20 million</td>
<td>116 (30.2) *</td>
</tr>
<tr>
<td>20-30 million</td>
<td>129 (33.6) *</td>
</tr>
<tr>
<td>30-50 million</td>
<td>100 (26.0) *</td>
</tr>
<tr>
<td>&gt;50 million</td>
<td>39 (10.2) *</td>
</tr>
<tr>
<td>Overweight or Obesity</td>
<td>175 (45.6) *</td>
</tr>
<tr>
<td>Heart disease</td>
<td>36 (9.4) *</td>
</tr>
<tr>
<td>Stroke</td>
<td>18 (4.7) *</td>
</tr>
<tr>
<td>Hypertension</td>
<td>76 (19.8) *</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>62 (16.1) *</td>
</tr>
<tr>
<td>type 2 diabetes</td>
<td>59 (15.4) *</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>19 (4.9) *</td>
</tr>
</tbody>
</table>

Data are expressed as † mean ± SD, †† median [IQR], *number (%)
Table 2: Oil consumption and brand preference of the studied participants for major types of oils

<table>
<thead>
<tr>
<th>Frying oil</th>
<th>Sunflower oil</th>
<th>Corn oil</th>
<th>Canola oil</th>
<th>Rapeseed oil</th>
<th>Mixed oil</th>
<th>Solid oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)*</td>
<td>302 (78.6)</td>
<td>233 (60.7)</td>
<td>43 (11.2)</td>
<td>28 (7.3)</td>
<td>11 (2.9)</td>
<td>12 (3.1)</td>
</tr>
<tr>
<td>Brand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A*</td>
<td>34 (11.3)</td>
<td>26 (11.2)</td>
<td>5 (11.4)</td>
<td>8 (28.6)</td>
<td>1 (9.1)</td>
<td>-</td>
</tr>
<tr>
<td>B*</td>
<td>124 (41.1)</td>
<td>94 (40.3)</td>
<td>18 (40.9)</td>
<td>10 (35.7)</td>
<td>2 (18.2)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>C*</td>
<td>38 (12.6)</td>
<td>54 (23.2)</td>
<td>3 (6.8)</td>
<td>3 (10.7)</td>
<td>1 (9.1)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>D*</td>
<td>7 (2.3)</td>
<td>5 (2.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E*</td>
<td>5 (1.7)</td>
<td>5 (2.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F*</td>
<td>5 (1.7)</td>
<td>6 (2.6)</td>
<td>3 (6.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G*</td>
<td>42 (13.9)</td>
<td>11 (4.7)</td>
<td>1 (2.3)</td>
<td>-</td>
<td>3 (27.3)</td>
<td>-</td>
</tr>
<tr>
<td>H*</td>
<td>2 (0.6)</td>
<td>1 (0.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I*</td>
<td>4 (1.3)</td>
<td>1 (0.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unknown*</td>
<td>41 (13.6)</td>
<td>29 (12.4)</td>
<td>13 (29.5)</td>
<td>6 (21.4)</td>
<td>4 (36.4)</td>
<td>2 (16.7)</td>
</tr>
</tbody>
</table>

Data are expressed as *number (%) or † median [IQR]
11 consumed oil brands reported by participants for main oils were coded in alphabetical order from A to K

Table 3: Oil consumption and brand preference of the studied participants for other types of oils

<table>
<thead>
<tr>
<th>Olive oil</th>
<th>Grapeseed Oil</th>
<th>Sesame oil</th>
<th>Animal oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>144 (37.5)</td>
<td>8 (2.1)</td>
<td>70 (18.2)</td>
</tr>
<tr>
<td>Annual use (kg)</td>
<td>4.6 [2.4-12]</td>
<td>2.2 [1.1-5.2]</td>
<td>6 [2.9-12]</td>
</tr>
<tr>
<td>Brand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imported edible oils</td>
<td>23 (16.0)</td>
<td>3 (37.5)</td>
<td>5 (7.1)</td>
</tr>
<tr>
<td>Home-made oil</td>
<td>17 (11.8)</td>
<td>2 (25)</td>
<td>42 (60.0)</td>
</tr>
<tr>
<td>A</td>
<td>24 (16.7)</td>
<td>-</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>B</td>
<td>3 (2.1)</td>
<td>-</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>C</td>
<td>4 (2.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>3 (2.1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Industrial oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not knowing</td>
<td>70 (48.6)</td>
<td>3 (37.5)</td>
<td>20 (28.6)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%)
4 consumed oil brands of other types reported by participants were coded in alphabetical order from A to D
Table 4: Factor associated with the type of Oil consumption in the studied participants by multivariate logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Cooking oil</th>
<th>Sunflower oil</th>
<th>Solid oil</th>
<th>Frying oil</th>
<th>Other oil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td>1.04 (1.01 to 1.07)</td>
<td>0.96 (0.94 to 0.98)</td>
<td>1.001 (0.97 to 1.03)</td>
<td>0.99 (0.96 to 1.02)</td>
<td>1.03 (1.01 to 1.06)</td>
</tr>
<tr>
<td><strong>Family size</strong></td>
<td>1.05 (0.84 to 1.30)</td>
<td>0.95 (0.79 to 1.14)</td>
<td>1.22 (0.99 to 1.51)</td>
<td>1.53 (1.19 to 1.96)</td>
<td>0.98 (0.81 to 1.18)</td>
</tr>
<tr>
<td><strong>Marital status, single (ref)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>0.61 (0.25 to 1.53)</td>
<td>1.42 (0.63 to 3.19)</td>
<td>1.08 (0.40 to 2.87)</td>
<td>0.44 (0.15 to 1.31)</td>
<td>0.47 (0.19 to 1.11)</td>
</tr>
<tr>
<td><strong>Education, under diploma (ref)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>1.45 (0.69 to 3.04)</td>
<td>1.05 (0.59 to 1.87)</td>
<td>0.37 (0.19 to 0.69)</td>
<td>0.71 (0.34 to 1.49)</td>
<td>2.01 (1.11 to 3.65)</td>
</tr>
<tr>
<td>Academic</td>
<td>3.26 (1.43 to 7.45)</td>
<td>0.89 (0.45 to 1.74)</td>
<td>0.27 (0.13 to 0.59)</td>
<td>0.68 (0.29 to 1.57)</td>
<td>2.65 (1.33 to 5.29)</td>
</tr>
<tr>
<td><strong>Job, housekeeper (ref)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>0.50 (0.24 to 1.03)</td>
<td>1.70 (0.89 to 3.23)</td>
<td>1.40 (0.64 to 3.06)</td>
<td>1.04 (0.50 to 2.18)</td>
<td>1.82 (0.95 to 3.49)</td>
</tr>
<tr>
<td>Self-employment</td>
<td>0.39 (0.12 to 1.24)</td>
<td>1.16 (0.53 to 2.58)</td>
<td>2.19 (0.95 to 5.08)</td>
<td>0.75 (0.31 to 1.82)</td>
<td>0.78 (0.36 to 1.72)</td>
</tr>
<tr>
<td><strong>Family income, &lt;2million (ref)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3 million</td>
<td>0.91 (0.45 to 1.82)</td>
<td>1.11 (0.64 to 1.93)</td>
<td>1.63 (0.89 to 2.99)</td>
<td>1.18 (0.61 to 2.29)</td>
<td>1.23 (0.71 to 2.14)</td>
</tr>
<tr>
<td>3-5 million</td>
<td>2.26 (1.11 to 4.59)</td>
<td>0.71 (0.39 to 1.32)</td>
<td>0.68 (0.32 to 1.42)</td>
<td>1.15 (0.54 to 2.47)</td>
<td>1.11 (0.59 to 2.06)</td>
</tr>
<tr>
<td>&gt;5 million</td>
<td>0.71 (0.25 to 2.04)</td>
<td>0.59 (0.26 to 1.37)</td>
<td>0.75 (0.26 to 2.20)</td>
<td>0.81 (0.31 to 2.14)</td>
<td>2.41 (0.95 to 6.09)</td>
</tr>
<tr>
<td><strong>Overweight or obesity</strong></td>
<td>1.001 (0.59 to 1.70)</td>
<td>1.24 (0.79 to 1.93)</td>
<td>0.93 (0.56 to 1.54)</td>
<td>1.18 (0.69 to 2.00)</td>
<td>1.06 (0.69 to 1.67)</td>
</tr>
<tr>
<td><strong>Heart disease</strong></td>
<td>0.89 (0.36 to 2.24)</td>
<td>0.96 (0.44 to 2.12)</td>
<td>0.73 (0.29 to 1.83)</td>
<td>0.31 (0.14 to 1.98)</td>
<td>1.09 (0.49 to 2.45)</td>
</tr>
<tr>
<td><strong>Stroke</strong></td>
<td>3.45 (1.45 to 10.27)</td>
<td>0.67 (0.23 to 1.90)</td>
<td>2.51 (0.78 to 8.06)</td>
<td>2.38 (0.45 to 12.68)</td>
<td>1.41 (0.45 to 4.45)</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>1.03 (0.52 to 2.02)</td>
<td>1.07 (0.60 to 1.92)</td>
<td>1.44 (0.75 to 2.78)</td>
<td>2.71 (1.17 to 6.29)</td>
<td>1.18 (0.65 to 2.15)</td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td>0.58 (0.27 to 1.28)</td>
<td>1.05 (0.56 to 1.98)</td>
<td>0.46 (0.20 to 1.05)</td>
<td>1.04 (0.47 to 2.31)</td>
<td>1.83 (1.09 to 3.79)</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>1.01 (0.47 to 2.16)</td>
<td>1.36 (0.71 to 2.60)</td>
<td>0.99 (0.47 to 2.10)</td>
<td>1.22 (0.54 to 2.79)</td>
<td>1.06 (0.54 to 2.07)</td>
</tr>
<tr>
<td><strong>Chronic kidney disease</strong></td>
<td>0.87 (0.25 to 3.09)</td>
<td>1.74 (0.57 to 5.28)</td>
<td>0.66 (0.19 to 2.28)</td>
<td>1.05 (0.27 to 4.04)</td>
<td>0.89 (0.34 to 2.52)</td>
</tr>
</tbody>
</table>

Data are expressed as Odd’s Ratio (95% CI). Cooking oils including corn, canola, rapeseed and mixed oils; Other oils including olive, sesame, animal and grapeseed oil.
Discussion

In the present study the profile of edible oils in a sample of Iran population in Shiraz was assessed. Our findings show that the daily households’ oil consumption was 110.5 grams. Frying (78.6%), sunflower (60.7%), and olive (37.5%) were the most frequent oils consumed by our studied households. Age, income, family presence of chronic disease, education, and family size were among factors associated with the type of oil consumption. 57.6% participants read the oil container labels, 28.9% do not understand label information. The level of fatty acids and resistance to heat in the most frequent brands of different types of assessed oils were in the standard range.

We find that daily use of oil consumption in our study population was 110.5 grams. This finding is lower than that reported for other study. Salehzadeh et al. studied 460 households in Sanandaj city, Iran, and reported that the mean oil consumption per household was 149.2925 grams(22). The difference between findings can be explained by different characteristics of studied population. Our study population were older and had higher education level than Salehzadeh et al. studied population. It is previously show that age and education are associated with edible-oil intake behavior(23). Salehzadeh et al. identified that consumed amount of oil was associated with higher education levels, however, they found that the households with higher education used sesame and olive oils significantly more than those households with low education levels consumed amount of oil was higher education levels(22).

Frying oil was the most frequent consumed oils in our study. Frying oil which is the most common consumed oil in Iran is vegetable and animal oil that is used to fry food at high temperatures. In our study all fatty acid profile of most of common consumed frying oil are in the standard range. The third common brand of frying oil which used by 12.6% of consumers contain myristic acid and SFA out of standard range. In a study by Abedi et al. (24), fatty acids of frequently consumed edible oils and fats marketed in Iran were assessed and they found that the highest and contents of SFA was seen in frying oils. Evidence show that myristic acid and SFA is associated with coronary heart disease. Where the replacement of...
myristic acid and SFA were associated with reduction of coronary heart disease risk\(^\text{(25)}\). Sunflower oil was the second most frequent consumed oils in our study. Also, all fatty acid profile of the most of common consumed sunflower oil are in the standard rang. Similarly, other studies reported that sunflower is the most consumed oils for cooking in India and Turkey\(^\text{(26-27)}\). Compare with the other oils, refined sunflower oil is one of the healthiest and cheapest oils. This oil is suitable for frying due to good thermal stability and high smoke point. In addition, because of ability to keep viscosity and consistency at lower temperature it can good to making salad. In the other hand sunflower oil is containing high proportion of polyunsaturated fatty acids\(^\text{(28)}\). Linoleic is one of the acids that is high in this oil. It is shown that presence of linoleic has hypocholesterolemia effect and can reduce the risk of cardiovascular disease. Also, this oil contains vitamins and natural antioxidants\(^\text{(29)}\). Olive oil was the third most frequent consumed oils in our study. The fatty acid profile of the two most of common consumed olive oil were in the standard rang. Home-made olive oil which used by 11.8% of consumers contains palmitoleic, oelic, linoleic, linolenic acids and SFA out of standard range. Similarly, olive oil reported to be the second most consumed oil in Turkey\(^\text{(26)}\). Olive oil contains high amount of triolein which is beneficial for health and has aging retarding effect\(^\text{(29)}\). In addition, olive oil known to be effective in the prevention of cardiovascular, hypertension, cancer, digestive system and nervous system diseases\(^\text{(29-30)}\).

Our results showed 57.6% of consumers read labels before making a purchase but 42.4% do not read the labels. Similarly, one study reported that 53.4% of the consumers read and 46.6% do not read the labels before purchasing\(^\text{(31)}\). Another study, show that 27% of consumers have never or rarely read food labels before purchasing\(^\text{(32)}\).

This study has some strengths and limitations to highlight. Strengths were as follow; a comprehensive assessment of the various common brands of oils was done. Other than participants oil consumption assessment, their attention to labels and their literacy in this regard were also evaluated. Also, for better precision, all questionnaires were completed by face- to-face interview by the main investigator. Our limitations were as follow; first, data collected for this survey is self-reported households’ consumption and used brands of edible oils in their daily diets. This can lead to an under/overestimation of consumption data and information regarding the used brands. Second, sample size may be not enough to generalize our results to all regions of Iran. Third, this study is limited to the one city, therefore because of the different cultural and economic characteristics, the results cannot be generalized to the other cities in Iran. So, multicenter studies with larger sample size must be down to more clarify households’ consumption and used brands of edible oils.

**Conclusion**

In summary, the present study revealed that frying, sunflower, and olive oils were the most frequent oils consume reported by studied households. We found that oils consumption among studied population decrease compare than the last year, these can be helpful to reduce the risk of chronic diseases as one of the main causes of overweight or obesity. The level of fatty acids and resistance heat in the most frequent brands of different types of assessed oils were in the standard range. These finding show that Iran policies targeting to control the fatty profile of the most of the edible oils were effectiveness, although, monitoring and updating information on the fatty profile of edible oils based on new evidence should be considered by policy makers. In addition, our other finding identifies that 42.4% of studied households do not read the oil container labels before making a purchase. These finding shows the need for learning programs to increase the households’ knowledge and awareness about the appropriate use of edible oil container labels.

**Conflict of interest:** All authors have no Conflict of interest

**Source of Funding:** This study is supported by Shiraz University of Medical Sciences, Shiraz, Iran.

**Ethical clearance:** The protocol of the study is approved by the Ethics Review Board of the Shiraz University of Medical Sciences.

**References**

2. Global Health Estimates 2016: Deaths by Cause,


Mitigation of the Biofilm Using Nanoparticles By Enhanced by D-alanine and D-proline

Fakhri Alajeeli
Lecture in Al-HIKMA University College –Department of medical laboratory-Baghdad –Iraq.

Abstract

The present study was undertaken to investigate the susceptibility patterns as antifungal and growth of certain D-amino acids inhibitory effects, includes D-alanine (D-ala) and D-proline (D-pro). The obtained results indicated that D-proline acid is potent mostly as antifungal, among tested D-amino acids, then followed by D-ala. The study aim was evaluating the of D-amino acids and nanoparticles against Candida albicans adhered cells and bio-films. Results showed that D-ala MIC value was 25 μg/ml, while in methods of TCP, (D-pro) MIC 50 μg/ml value. Nanoparticles (lithium and silver) and amino acids (D-pro and D-ala) effect in preventing cells adhesion on polystyrene surface and mature bio-film inhibition was studied. Highest inhibition was obtained at D-pro concentration 50 μg /ml, while the lowest at concentration D-ala 25 μg /ml against mature bio-film and cell adhesion.

Keyword: D-aminoacids, D-alanine, D-proline, bio-film, nanoparticles

Introduction

Candida considered as part of human body normal flora that colonize different anatomical sites i.e. digestive tract, oral cavity, skin and vagina 1. Candidacies are the cases where local change in the environment or debilitation of host take place and encourage overgrowth of Candida resulting in candida infection 2. Candida species pathogenicity is reasoned to certain factors being virulence, i.e. adherence, evades host defenses ability, bio-film formation (on medical devices and on host tissue 3. Candida species ability for forming bio-films drug-resistant is significant factor in their human disease contribution. Drug resistance progression within Candida bio-films correlated with a parallel elevation in process of maturation4. Resistant strains increase necessitates new targets search for new agent as antifungal 5.

In all life kingdoms, amino acids are basically found in L-enantiomeric form. Nevertheless, D-amino acids significant amounts are bacteria produced; as major D-amino acids producer at ecosystem 6. In peptidoglycan cross-linking 7. In recent years, D-amino acids are releasing via various species of bacteria in the growth stationary phase and behave as agents controlling modification and assembly of cell wall 8.

Nanoparticles for many years have been known for its importance as antimicrobial broad-spectrum activity against bacteria of G+ and G-, protozoa, certain viruses, and fungi 9, including antibiotic-resistant strains 10. Ag, as agent being antimicrobial, is utilized in burn treatments, creams, wound dressings, and as coatings on various devices in medicine 11.

Material & Method

The Candida isolate from different sources were isolated and identified. All samples were cultured on agar of Sabouraud Dextrose then were aerobically incubated for 24-48 h at 37 ºC 12. Isolated of Candida were identified based on features of morphology on culture media, formation of germ tube, formation of Chlamydospore, CHRO Magar 13 and along Vitek 2 compact system utilizing 14. Bio-film formation by Candida spp. isolates 15.

Nanoparticles preparation

Nanoparticles in de-ionized water were insoluble based on producer 16.

Amino acids (D-ala and D-pro) Minimum
Inhibitory Concentrations Determination

Preparation of D-ala and D-pro was done for determining planktonic cells MIC. Solution of 1 M as stock for every amino acid was prepared in D.W. These solutions were filter-sterilized via membranes passage through of 0.45 µm (Billerica, MA. USA). Such were prepared to obtain various molarities for every amino acid, beginning with 100 µg/ml and following dilution series were preformed with the media till concentrations point ending. Tests of MIC were preformed in ninety six plate’s flat bottom micro-titer (TPP, switerland). With hundred ml broth of Mueller-Hinton, every test well was filling. Addition of 100 µl of solution as stock to 1st test well and mixing, then dilutions series was applied across the plate after that ten µl of microorganism liquid of (Candida albicans isolate) was applied for inoculating every plate well of micro-titer to reach a final size of inoculums $1.5 \times 10^8$ CFU/ml well with culture overnight. Fungi inoculums along broth of Mueller-Hinton but with no a.a. treatment were considered as positive controls growth, while D-amino acid without inoculums considered as negative controls. Under same conditions of experiment, all wells as control were prepared and incubated where plates incubated at 37°C for 48 hr. Wells were tested via naked eye for growth of microbes. MIC values were described as the lowest concentration of D -amino acid inhibiting microbial growth of 80%, comparing to positive and negative controls, and growth of microbes in wells as turbid was detected. Determination of MIC in triplicate was done.

Effect of combination of (D-ala and D-pro) and Nanoparticles(Ag and LiO₂) on the adherent cells and mature biofilm as treatment.

Bio-film assays formation were done utilizing 96 micro-titer well plate, according to protocols of with minor modifications. Briefly C. albicans isolate were cultured overnight in SD Broth and dilution preformed to the resulting culture as 1:100 (SDB + 1% w/v glucose). Suspensions of cells (two hundreds µL) were poured in every well and incubation at 37°C for 48 h was preformed, after bio-film formation for 48 h. Every well of plate micro-titer was filled with hundred ml of media and hundred µl of D-pro fifty µg/ml and D-ala twenty five µg/ml, whereas well of control no amino acids were added. Every concentration for each tested amino acid was triplicate assayed. Incubation for plates was preformed for 24 h at 37 °C. The fungi as planktonic were taken away via dish shaking over tray of waste loaded by sterile D.W. Solution (crystal violet) of 0.1% v/v subsequently was added to every well where plates were stand for staining at room temperature for 10 min. Solution (crystal violet) was taken away through plate submerging in tray of water. Inverting to plates was done and on paper towels was topped to take away liquid as excess and left for air drying. Ethanol of 95% v/v applied to wells stained at room temperature for 10 min for dye solubility. Suspension of fungi in every well was well mixed and its OD was read at 490 nm at micro-plate reader. Also the effect of mixing 25 µg/ml of D-ala with twenty five µg/ml of Lio2, fifty µg/ml silver and fifty µg/ml D-pro with twenty five µg/ml of Lio2, fifty µg/ml silver were prepared by loaded 50 µl of each amino acid and nanoparticle with 100 µl of medium after biofilm formation for 48 h and the other steps were the same.

Statistical Analysis

The experiments in the current study was designed as factorial experiments (2×6), every combination treatment was replicated 3 times. By SPSS 2010 program, Duncan Multiple test and ANOVA were applied to show differences among means at (p < 0.05).

Result and Discussions

D-ala and D-pro Minimum Inhibitory Concentrations (MIC) Determination

Table (1) expresses values of MIC for D-amino acids in respect to isolate of C. albicans. D-proline MIC was 50 µg/ml. The MICs for D-alanine was 50 µg/ml

Table (1): (D-ala and D-pro) MIC means against isolate of C. albicans.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Plate tissue culture (µg/ml) (MICs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-alanine</td>
<td>25</td>
</tr>
<tr>
<td>D-proline</td>
<td>50</td>
</tr>
</tbody>
</table>
values of MIC for D-lysine and D-alanine were 18 and 39 µg/mL\(^{-1}\)\(^{20}\). In other study,\(^{21}\) recorded D-isomers antifungal activities, L-isomers with no detectable activity against species under test (MIC more than two hundreds µg/µL). D-lys as one among D-amino acids tested revealed activity as highest against *Candida albicans* with a 6 µg/µL value of MIC. Least species susceptibility was *Candida glabrata* of treatment to mostly D-amino acids. Growth of *Candida krusie* was extensively mostly inhibited via D-lys then by D-ala, while considerable D-ser higher concentration was required for growth inhibiting all species tested of *Candida*. It is obvious, D-pro exhibit no significant activity as anticandidal against tested species.

Antimicrobial peptides affect dendrites’ cells recruitment and inflammation, thus immune response modulating\(^{22-23}\); some AMPs can induce apoptosis\(^{24}\). Limited studies were reported regarding ultra-structural level of D-amino acids effect with fungal cells. It was proposed that *C.albicans* exposure to D-amino acids yield membrane changes significant, cell surface pits formation and ultimately poress formation and death of cell\(^{25}\).

**Effect of combination of (D-ala and D-pro) and Nanoparticles(Ag and LiO\(_2\)) on the adherent cells and mature biofilm as treatment.**

Highest mature bio-film and adherent cells were (70.714 and 52.848)% in D-Pro(conA) acid of 50 µg/ml with high differences of significant comparing to other concentrations which of no significant against mature bio-film and planktonic cells. Although the lower percentage value (52.509) of mature bio-film and adherent cells, inhibition was noticed for isolate of *C.albicans* at combination D-amino acids (Ag50+AL25) µg/ml of sub-MIC and sub-MIC of nanoparticles(Ag), respectively, Tabel(2),fig(1)

**Table (2): Effect of combination of (D-ala and D-pro) and Nanoparticles(Ag and LiO\(_2\)) on the adherent cells and mature biofilm.**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Treatments</th>
<th>Amino Acid</th>
<th>Mean (%) ± S.d.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Con.proline( P 50)</td>
<td></td>
<td>66.215 ± 6.879</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Con. Alanine(AL25)</td>
<td></td>
<td>64.708 ± 11.044</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Con. Li25+P50</td>
<td></td>
<td>64.041 ± 11.933</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Con. Li25+AL25</td>
<td></td>
<td>63.429 ± 13.778</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Con. Ag50+P50</td>
<td></td>
<td>59.786 ± 13.554</td>
<td>Ab</td>
</tr>
<tr>
<td></td>
<td>Con. Ag50+AL25</td>
<td></td>
<td>52.509 ± 14.120</td>
<td>B</td>
</tr>
<tr>
<td>LSD</td>
<td>P ≤ 0.05</td>
<td></td>
<td>9.003</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Amino acid. adherent cells</td>
<td></td>
<td>70.714 ± 7.675</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Amino acid Bio</td>
<td></td>
<td>52.848 ± 8.761</td>
<td>B</td>
</tr>
<tr>
<td>LSD</td>
<td>P ≤ 0.05</td>
<td></td>
<td>5.198</td>
<td></td>
</tr>
<tr>
<td>L.S.D.</td>
<td>Least Significant Difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Significant Difference at p ≤ 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non Significant Difference</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Observed report showed that in assay of bio-film, both L- and D- Asp, Glu, and Cys enantiomers with 40 mM concentration inhibit significantly formation of *S. mutans* bio-film, where other A.A. not. Asp and Cys anti-bio-film activity were higher than of Glu. At 20 mM concentration, Glu significantly did not prevent formation of bio-film. The reported that D-aspartic acid inhibit biofilm formation on tissue culture plates similar to, which observed that the high concentration above (10mM) inhibited the growth of *staphylococcus aureus* planktonic cells. In recent years, D-amino acids proved to have significant roles in regulating disassembly and bacterial bio-films formation, and might express bio-film prevention general strategy. Hassan et al. (2013) stated that results obtained revealed the Ag-NPs MIC50, Grisofulvin and Itraconazole on *T. mentagrophytes* and *C. albicans* which were (8±0.18) µg/ml, (4±0.25) µg/ml and (2±0.10) µg/ml respectively, on *C. albicans*. Such result confirms requiring investigation agents as alternative or drugs combinations, i.e. SN use with drugs as antifungal. Combinations use along lower concentrations drug able to elevate efficacy of drug and minimize drugs adverse effects. Based on SN effect yeast cells via membranes attacking, hence membrane disruption potential. Such authors noticed, via microscopy as transmission electron, pits formation on the *C. albicans* membrane surfaces and ultimately pores formation and subsequent death of cell.

**Ethical Clearance**- Taken from Baghdad University - college of science -Biology department .committee

**Source of Funding**- Self

**Conflict of Interest** - (Nil).

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Detection the Serogroups of Escherichia coli isolates from Urine sample of UTI patients

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Aims: Determining of E.coli serogroups which isolation from UTI patients

Patients and Methods: the study was conducted between September 2019 to April 2020 and they was collected 40 sample from patient how are diagnosis with UTI during this study .

Results: From the UTI patients individuals, 40 strains were identified in which O serogroups showed 8(30%) and 2 (20%), respectively, of occurrence in those strains. Moreover, the enterohemorrhagic E. coli (O157 H7) and the enteroaggregative E. coli (O44 H7) were detected in two strains of the UTI-based urine samples only. For the antibiotic resistance properties of the biofilm positive producers isolated from the UTI urine samples, multidrug resistance was revealed in 60% of those isolates; however, only 20% of the biofilm negative producers from the UTI urine samples showed the MDR characteristics. Biofilm was identified in 75% of the UTI urine. For the antibiotic sensitivity test, the results demonstrated that the sensitivity rates to Imipenem, Amikaci, gentamicin, and tobramycin were 90%, 100%, 65%, and 40%, respectively, in the UTI strains.

Conclusion: The herein study provides thorough data about the Escherichia coli based biofilm,

Keywords: Biofilm, E. coli, UTIs, Resistance of antibiotics, uro-pathogenic Escherichia coli.

Introduction

Escherichia coli is a well-known resident of the human and animal gastrointestinal system. E. Coli establishes a significant symbiotic connection together with its habitat, performs a significant act in encouraging the stabilization and maintenance of ordinary gastrointestinal homeostasis of the macrobiotic. For the commensal property, E. coli is contained in the gastrointestinal tract causing illnesses only in occasion cases. However, even E. coli of non-pathogenic-commensal natures can induce illnesses in immunosuppressed or in the GIT-barrier-based defected patients 1 Virulence factors are acquired by these strains playing very important roles in providing these bacteria with specific properties to perform intestinal-niche-based adaptation and generate illnesses. These properties are obtained by the bacteria via genetic materials transferring for other bacteria or environment such plasmids, horizontal gene transfer, and/or bacteriophages. E. coli can generally be categorized into enteric/diarrhegenic E. coli or extra intestinal E. coli . More specifically, E. coli can be classified into six “pathotypes” of enterohemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), entero aggregative E. coli (EAEC), enterotoxigenic E. coli (ETEC), diffusely adherent E.Coli (DAEC), and enteroinvasive E. coli (EIEC) 2

One of the more often recognized infections in humans caused by uropathogenic Escherichia coli (UPEC) are urinary tract infections (UTI) that include pyelonephritis and cystitis. The UTIs can rely on various factors such as host immune responses, anatomical properties, and the bacterial virulence. For the E. coli to induce an infection, the bacterium has to have the abilities performing host-cell-related adhesion, tissue bacterial colonization, intracellular invasion (in some bacteria), bacterial proliferation, and blood-dependent dissemination to different body systems. Bacteria can be present in the urine as a result to colonization generating no symptoms as it is named asymptomatic bacteriuria 3. Asymptomatic bacteriuria based illnesses may not require medical attention, in fact, it can provide the immune system with proper defense mechanisms
against other strains of *E. coli*[^4]. UTI treatment with antibiotics can be successful; however, the problem of resistance by the bacteria to antibiotics is increasing leading to recurrent occurrence of the UTIs especially in the presence of biofilm formation and virulence factors[^5].

The aim of this study is determination of serogroups of *E. coli* which are isolated from UTI patient.

### Patients and collecting samples

There were 150 urine samples were collected from patients with the symptoms of urinary tract infection who had been admitted at Al-Yarmouk teaching hospital in Sept 2019 tell April 2020. To confirm infection with *E. coli*, the samples were cultured in the EMB Agar and Blood Agar and were identified by the Gram stain, Indole test, and Citrate and MR-VP tests. The laboratory criteria of acute urinary tract infection with *E. coli* included one positive culture of colonies with a minimum number of 105 colonies per 1 ml of urine collected in the urology department of Al Yarmouk teaching hospital in Baghdad between September 2019 tell April 2020.

### Serotyping

According to Liu et al., 2015. The test included O and H antigenic based identification using an antibody (polyvalent and monovalent)-detected agglutination test. The test was performed using methods concluded from.[^6]

### Antibiotic sensitivity test

The antibiotic sensitivity test depended on Kirby Bauer disc diffusion method.

### Biofilm formation

According to Nourbakhsh et al., 2016 the ability of *E. coli* isolates to produce biofilms, biofilm test was performed in laboratory based on the Micro titer Plates Assay as follow as:

The micro titer plate method was used for evaluating the formation of UPEC biofilm. The Micro titer Plates Assay method, such as ELISA, the color-producing chromogenic in this technique is fuchsine, whose color intensity is directly related to the concentration of biofilm.[^7]

### Statistics

BM SPSS software v20.0. (Armonk, NY: IBM Corp) was used to process the observed data. Chi-square and Fisher’s exact tests were performed. Data, sometimes, were displayed as numbers and percentages (%). The null hypothesis was not followed when *p* is less than 5%.

### Results

#### Serotyping

From the UTI patients and healthy individuals, 40 and 20 strains, respectively, were identified in which O serogroups showed 7 (35%) and 3 (15%), respectively, of occurrence in those strains. Moreover, the enterohemorrhagic *E. coli* (O157 H7) and the enteroaggregative *E. coli* (O44 H7) were detected in two strains of the UTI-based urine samples only, figure 1

Figure 1: Total of serotype percentages of *E. coli* isolation from UTI patient
Antibiotic sensitivity test

For the antibiotic resistance properties of the biofilm positive producers (BPPs) isolated from the UTI urine samples, multidrug resistance (MDR) was revealed in 60% of those isolates; however, only 20% of the biofilm negative producers (BNPs) from the UTI urine samples showed the MDR characteristics. For the antibiotic sensitivity test, the results demonstrated that the sensitivity rates to imipenem, amikacin, gentamicin, and tobramycin were 90%, 100%, 65%, and 40%, respectively, in the UTI strains, figure 2

Discussion

UTIs are well-known cases of illnesses with high incidence rates in humans, and UPEC plays an important role in the etiology of these infections. For those bacteria, a wide range of virulence factors are considered as critical components of the UPEC providing those strains with different capabilities such as adherence to the host cells enhanced by some factors; for example: adhesins that play huge roles in bacterial attachment to epithelial cells of the urinary system.

For the O serogroup, UPEC-associated O-serogroups were detected in high rates, 60%. The current work revealed that 2 (10%) of the strains isolated from the urine samples were not placed in a certain serogroup. This agrees with who found that in their results about (28%) of their strains were unidentifiable in a certain serogroup. have also found that 25.5% of their recognized strains were from unknown serogroups. Moreover, Gilbert NM, Lewis AL, observed the same rate, 25%, of the unidentifiable strains.

Moreover, the enterohemorrhagic E. coli (O157 H7) and the enteroaggregative E. coli (O44 H7) were detected in two strains of the UTI-based urine samples only. This was also reported by who found that strains of UPEC from UTIs but not the stool were able to produce UTIs in murine.

For biofilm formation, this bacterial mechanism is considered as a defense strategy to defeat body boundaries and escape body immune system components. The biofilm was recognized in human infections in a rate of 60%.

Ethical Clearance- Taken from Baghdad University –College of science –Biology department committee

Source of Funding- Self

Conflict of Interest -nil

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Management of Non-Vital Teeth Discoloration with the Internal Bleaching: A Case Report

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Abstract

Tooth discoloration due to intra coronal bleeding after trauma can cause aesthetic disturbances and appearance. Internal bleaching is a non-invasive method to restore tooth color which is performed after endodontic treatment by placing a strong oxidizing agent in the pulp chamber. Aim of this study was to report the success of internal bleaching procedure to restore discolored teeth due to trauma. A 44-year-old female came to Conservative Dentistry Universitas Airlangga with chief complaint of the upper right insisor tooth looks darker than the adjacent teeth. The patient had fallen 10 years ago from her motorcycle and her tooth had been painful and then the pain disappeared. In intra oral clinical examination, it showed that tooth 11 had discoloured, vitality test of tooth 11 showed no response to electric pulp test. Radiographic view showed that radiolucen in periapical area and no fracture was found. At the first visit, treatment of single visit root canal using rotary instrument and gave dressing calcium hydroxide. A week later on the second visit, internal bleaching with hydrogen peroxide 35% was done. After bleaching, calcium hydroxide was applied to neutralize the oxidizing agent and then re-evaluated for the color. At the third visit, it showed that the tooth colour was obtain as desire from C3 to A3 (Vitapan Classical). The internal bleaching procedure on the traumatized tooth was the best option to restore the function and aesthetics according to the original tooth colour and also provided benefits in terms of saving time, lower price, and maximum results.

Keywords: non-vital teeth, internal bleaching, discoloration

Introduction

Discoloration of teeth can be caused by intrinsic, extrinsic or a combination of both. The occurrence of discoloration is caused by the incorporation of intrinsic chromatogenic materials into dentine and enamel during odontogenesis or after the eruption of teeth¹. The intrinsic cause of discoloration in pre-erupted teeth is the provision of tetracycline or high number of fluoride exposure. In addition, it also can be caused by hereditary diseases such as amelogenesis imperfecta and dentinogenesis imperfecta². After the eruption of teeth, the main cause of discoloration is the pulp necrosis, the deposition of the blood component to the dentine tubules after trauma or pulpectomy, and secondary dentine deposition due to aging or iatrogenic²,³. Foods and beverages such as coffee, tea, red wine, carrots, oranges, and tobacco are primarily responsible for extrinsic stains³.

Teeth whitening offers a conservative and aesthetic solution and is an option because it is not invasive, such as crowns or porcelain veneer. The non-vital teeth whitening technique puts the oxidizing agent inside the pulp chamber in direct contact with dentine. Chemicals which are often used as bleach are hydrogen peroxide 35%⁴. Walking bleach technique is a bleaching method...
by placing the active ingredient in the pulp chamber, followed by closing the tooth cavities. The results obtained depends on the etiology of discoloration and for a satisfactory result bleaching can be carried out for two to four times the application\textsuperscript{4}. The case report aims to demonstrate the success of non-vital teeth whitening that has discoloration after the endodontic treatment performed using a walking bleach technique with a good prognosis and without side effects.

**Case Report**

A 44-year-old female came to Conservative Dentistry and Endodontics Clinic Airlangga University with chief complaint of the upper right incisor tooth looks darker than the adjacent teeth. Patient had fallen 10 years ago from her motorcycle and her tooth had been painful and then the pain disappeared. The patient wants her tooth colour same as the adjacent teeth. Patient does not has a history of systemic diseases.

Extra oral examination shows symmetrical face, no abnormalities of the lips, the left and right mandibular glands are unaffected and painless. Intra-oral examination showed that crown of tooth 11 is darker in colour than the adjacent teeth. Hygiene of the oral cavity is good, it shows an overview of radiolucency on periapical area. Examination of negative percussion test, negative bite test and negative palpation.

Radiographic view after endodontic treatment shows the radiopaque depiction of the crown to the apex which means that it fills the hermetic root canal. There is widening of lamina dura, alveolar bone resorption and radiolucen in periapical area which are smaller than before endodontic treatment.

On the first visit, the patient was given an explanation of the procedure to be performed. The cost of treatment, side effects can occured, possible outcome to be achieved, failures and complications about the use of bleaching material understood by the patient and then carried out the signing of informed consent. The next step was to do single visit root canal treatment using rotary instrument and given dressing calcium hydroxide.

A week later on the second visit, internal bleaching with hydrogen peroxide 35% was carried out for 7 days. Previously, it was carried out tooth colour recording before treatment and intra oral photographs. Previously, the tooth was cleaned using a rotary brush and pumice powder to obtain the actual colour. The tooth colour was matched using Vitapan classical shade guide. In this case, the initial colour was C3 and the colour which want to achieve was A3 colour.

The working area was isolated with rubber dam and coronal access was carried out by reopening the temporary patch using a rounded diamond bur. The root canal filler material was ejected to a depth of 2 mm below the CEJ by using a hot plugger that has been given a stopper to provide a place for cervical seal material. The depth of filler intake was confirmed again using periodontal probe. Tooth 11 cavity was irrigated with sterile aquadest until clean and dried. The cervical seal was made by applying 2 mm thick glass ionomer cement following the cervical stripe outline. The resultant shape from a facial view is the “bobsled tunnel” outline.

The outline from the proximal view resembles a “ski-slope” (Figure 1, 2).\textsuperscript{5} After the cervical seal dried, the bleaching material in the form of hydrogen peroxide gel 35% (Opalescence Endo, Ultradent Product Inc., USA) was applied to the labial part, then the pulp chamber was given with cotton pellet and covered with glass ionomer cement. The patient was instructed to control 1 week later.

On the third visit, a subjective examination was conducted, it was no complaints from the patient. Objective examination showed that tooth discoloration from C3 to A3 (Vitapan Classical). Two weeks later, the tooth was restorated by composite materials which in accordance with the tooth colour.

On the third visit, a subjective examination was conducted, it was no complaints from the patient. Objective examination showed that tooth discoloration from C3 to A3 (Vitapan Classical). Two weeks later, the deposition was carried out by composite materials which in accordance with the tooth colour.
Tooth staining especially in the anterior teeth can interfere with a person’s appearance. In addition to invasive treatment, such as the making of crowns or veneer, tooth whitening or bleaching can be an alternative treatment. Contrary to the making of crowns or veneer, tooth bleaching is a relatively non-invasive treatment. The tooth bleaching which has been carried out before root canal treatment can be done with internal bleaching treatment. In this case, the tooth discoloration in the tooth 11 is caused by intrinsic factors.

Necrosis in the tooth 11 is caused by trauma that occurred about 10 years ago. Trauma to the tooth causes intra pulpa bleeding and erythrocytes lysis. Blood or blood components that bathe the pulp chamber will enter the dentinal tubules diffusely, resulting in a discoloration of the tooth crown. The discoloration which occurs relates to how long the pulp has become necrosis, the longer the colored compounds are in the pulp, the greater the degree of discoloration.

Bleaching is a more conservative alternative
treatment than making of the crown and labial veneer in the stained teeth. The chemical process in bleaching occurs when the whitening material is applied to the teeth, usually using oxidizing materials such as hydrogen peroxide, chlorine or sodium hypochlorite. Although the bleaching process is a complex chemical process, the basic principle of the majority of bleaching processes is the step-by-step oxidation of the dye with decomposition. Bleaching material is oxidizing, reacting to the organic structure of a hard tooth tissue, pigments which have long chains slowly degraded into simple molecules such as carbon dioxide, oxygen and water which have a brighter colour, inorganic molecules are not broken down, the reduction-oxidation reaction which occurs in the bleaching process is known as redox reaction. In general, unstable peroxide becomes unstable free radicals. These free radicals oxidize other molecules.

Hydrogen peroxide is a strong oxidizing agent. The use of high concentration of hydrogen peroxide must be careful because it is unstable thermodynamically and can explode, because it must be placed in the refrigerator and stored on a dark container. Bleaching materials can be applied using the plastic and plugger instruments and must be replaced every 3-7 days. Usually it takes 2-4 sessions depending on the intensity of discoloration. The patient must be reminded to pay attention to her teeth everyday so as not to overbleach. When this material is in contact with teeth, hydrogen peroxide will lose and penetrate into the surface of enamel and dentine due to the relatively low molecular weight of peroxide (30 g/mol). These reactive molecules attack dark chromophore molecules and long chains and break down these molecules into smaller, more colourless, and more diffuse molecules.

The success of the intracoronal bleaching in endodontically treated teeth depends on the etiology, the precise diagnosis, and the selection of appropriate bleaching techniques. Walking bleach technique is chosen because it takes a shorter time, safer and more comfortable for the patient. Internal bleaching indications on the teeth that have been treated endodontically are: discoloration in the pulp chamber, dentine discoloration, discoloration which can not be lost with extracoronal bleaching. Internal bleaching contraindications: superficial discoloration, enamel formation with defects, severe dentine loss. The cervical seal/barrier used is the glassionomer to cover endodontic obturation. The coronal height closes the dentinal tubules and corresponds to the external epithelial attachment. The barrier thickness is approximately 2 mm, and is located 1 mm below the cemento-enamel junction.

This protection is carried out to prevent the occurrence of internal bleaching side effects in the form of external root resorption. Resorption occurs because of chemicals diffused through the dentine tubules and cementum defects. The leakage which occur can cause cementum necrosis, inflammation of the periodontal ligament so that eventually root resorption occurs. The application of bleaching material leads to denaturation of dentine protein with oxidizing agents and pH acid which induces foreign body reaction. A low pH of bleaching material can damage tissues due to the optimal acidic environment for osteoclas activity results in the root resorption.

In this case, restoration after internal bleaching using a composite resin. The remaining peroxide of the bleaching material, especially hydrogen peroxide can affect the strength of composite bonding, so it is advisable to wait several days after bleaching before the composite restoration is performed. The placement of calcium hydroxide in the pulp chamber for several days is useful for buffering acidity caused by bleaching material.

**Conclusion**

Internal bleaching procedures for traumatized teeth was the best option to restore the function and aesthetics according to the original tooth color.

**Funding:** None

**Conflict of Interest:** None

**Ethical Permission:** Approved

**References**


5. Bahuguna N. Cervical root resorption and non vital bleaching.


The Relationship between Antithyroid Antibody and Pregnancy Outcome in Invitro Fertilization and Embryo Transfer

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Abstract

Background of the study: One type of assisted reproductive technique is intracytoplasmic sperm injection (ICSI) Despite use ofintracytoplasmic sperm injectionICSI it remain has low birth rate rate due to failure there are many markers associated with poor predict the outcome one of these factor effect outcome intracytoplasmic sperm injection ICSI presence of antithyroid antibody ineuthyroid patient

Aim of study: To examine the effect of antithyroid antibody on pregnancy results following the in vitro fertilization and fetus transmit

Methods and patients: Cross section study conducted at AL-Sader infertility center a total of eighty one Iraqi women were enrolled in this study of them 22 positive antithyroid antibody, 59 negative antithyroid antibody were undergone intra-cytoplasmic sperm injection ICSI in Al-Sader infertility center. The group of the study involved only euthyroid women outwardly any clinically showed autoimmune illness.

Result: there was higher significant difference p value<0.004 in fertilization rate number of available embryos p value<0.003 and number of transfer embryos p value< 0.001 among study group which was higher in group of women with antithyroid negative followed by group with antithyroid positive in infertile euthyroid women Conclusion: patients with antithyroid antibody associated with poor pregnancy outcome in infertile euthyroid women undergoing intracytoplasmic sperm injection ICSI

Keywords: antithyroid antibody, pregnancy outcome, invitro fertilization, embryo transfer

Introduction

The definition of Subfertility when the husband and the wife couldn’t conceive following one year of unprotected sex affair (and there is no other cause, such as breastfeeding or postnatal amenorrhea) (1). It affects 12 –15% of all couples (2). Subfertility developed due to many factors and cases including both male and female factors. It classified to primary subfertility and Secondary subfertility (3). It has been estimated that subfertility affects 12-15 percent of all couples (4), where seventy percent struggle with initial subfertility, i.e. with no conception in the past and theirt percent secondary subfertility when the couple had conceived previously and whatever the outcome (5). The couple suffering infertile are advised to be tested for the elements that might be impairing fertility. The results of these measurements could be used as this information by the specialists of infertility to advise the husband and the wife regarding the possibilities of subfertility etiologies and to provide special plan for treatment for the special treatment of the couple. Its crucial to notice that the
husband and the wife can have many influences that contribute to subfertility; thus, a full primary diagnostic test must be be taken to find the main subfertility’s causes, the couples evaluations is performed on the same time, the treatment, evaluation, and recognition of subfertility makes the partners stressed and worried. The emotional state of the couple should not be ignored by the clinicians which may include marital discord, anxiety, anger, and depression. Information must be informative supportive.

Treatment of subfertility included both male and femal and include medical and surgical approaches. The conception of Assisting is the advice of normal conception through various forms of science research. The advancement of the enhanced approaches, specifically in forms of stimulations of controlled ovarian and ovulation induction, enabled the success process of anovulatory female treatment. Assisted conception includes three primary types; injection of intracytoplasmic sperm, insemination of intrauterine, and in vitro fertilisation. Previous studies showed some links between Female infertility and the thyroid dysfunction, therefore we tried in this work to examine the influence of antithyroid antibody on pregnancy results utilizing the technique of embryo transmit and in vitro fertilization among Iraqi women with subfertility.

Methods and patients

This study was planned as a prospective study performed in Al-Sadder Teaching Hospital/fertility center, Najaf, Iraq during period from 20th March till 31st December 2019. These patients were eightyone subfertile couples who were underwent Intracytoplasmic sperm injection

Inclusion criteria:

1. Age ranged from eighteen to forty-five years
2. The female has not been through treatment of adjuvant like glucocorticoids and thyroid hormones at the the research is being taken.
3. Normal TSH
4. Negative for LAC “lupus anticoagulant” and ACA “anticardiolipin antibody” before the start of the induction of ovulation
5. Exist of morphologically normal spermatozoa within sperm (male factor)
6. Normal utrine cavity

Exclusion criteria

1. Female partner with thyriod dysfunction
2. Female partner with hyperprolactinemia
3. Azoospermia
4. Confirmed endometriosis.

All women were asked to be seen at cycle day 2 (CD2) to evaluate if she was suitable for ICSI program, the history was taken from the patients included name, age, menstrual history, type of subfertility whether primary or secondary, duration and causes of subfertility, history of previous IVF and its outcome, family history, drug history. Examination had been done to each patient including body weight and height. The Body mass index (BMI) measured as individual body weight in Kg divided by square of her height in meters. Trans-vaginal ultrasound (TVUS) scan was performed to all patients using vaginal probe (5-7 MHZ). The baseline scan done to measure the endometrialthickness(mm), number of antral follicles (AFC), check the uterus and ovaries for any pathologies like fibroid, polyp, or ovarian cysts. All women sent for hormonal assay include FSH, LH, estradiol, Thyroid stimulating hormone (TSH), prolactin, anticardiolipin antibody, lupus anticoagulant and antithyroid antibody. Also routine screening blood tests of both partners for human immune deficiency virus (HIV), hepatitis B virus, (HBV) and hepatitis C virus (HCV). Before treatment, all women partners sent for seminal fluid analysis

Controlled ovarian hyperstimulation protocol

Using of one of following protocols in study.

1. Agonist GnRH protocol (short protocol)

GnRH agonist has been initiated by giving 0.1mg/day of triptoreline (decapetyl) from the cycle day two. The gonadotropins are started from the cycle day three with one of the following drugs: Recombinant FSH or HMG and doses according to patient till the follicle maturation monitoring by transvaginal ultrasound once
the follicles (dominant follicle) were reached diameter 18 or more, trigger was given to the patient. Ovum pickup was done using transvaginal ultrasound at 34 hours after trigger was given in dosing differed, depending on the age of patient and the response to stimulation.

2. Agonist GnRH protocol (Long protocol)

The agonist GnRH is given in late luteal section at cycle day 21 of preceding cycle. When the pituitary is down regulate. Transvaginal ultrasound was done to follow the maturation of follicles then added HCG, a minimum 3 follicles turned to be diameter 18 or more then ovum picked up after 34 hours.

3. Antagonist protocol:

On antagonist protocol gonadotrophin (human menopausal gonadotropin, recombinant FSH) started at day 3 of cycle, antagonist regime by used cetrotide (0.25 mg) starting after a week or at the time the dominant follicles reach the with a diameter of 14 mm. Once the follicles were have reached diameter 18 or more, trigger was given to the patient. Ovum pickup was done using transvaginal ultrasound at 34 hours after trigger was given in dosing differed, depending on the age of patient and the response to stimulation.

Ova pick up (OPU): Oocyte collection was performed 34 to 36 hours after trigger given; the procedure of oocyte retrieval was performed under general anesthesia. Under ultrasound guidance, aspiration of follicles done by using thin needle (REPRO Line, Germany) the researchers aspirated the fluid of follicular using gentle suction. The aspirated fluid then sent immediately to laboratory to be tested using a microscopy by embryologist to detect the oocytes and then the collected oocytes were transferred into culture medium in incubator. After that denudation process was performed by phenol red, the embryologist scored the oocytes using the inverted microscope, also he noted the maturation phases of the oocytes. Meanwhile sperm preparation was done; semen specimen was obtained after 3-5 days of the sexual abstinence in labeled standard sterile disposable plastic container at the day of pickup. Semen analysis was evaluated using the criteria of WHO pre and post the preparation process of semen. The preparation of semen included rinsing from seminal plasma, leukocytes, and bacteria. this approach can remove prostaglandins that cause uterine contraction.

ICSI: Intracytoplasmic semen injection was done on the whole oocytes which are morphologically intact that thrusted out the polar body number one (metaphase II). The procedure ICSI was as follow, a spermatozoon with single motile was chosen and immobilized thru pushing tail among dish’s bottom and the 33 microneedle. After that, the tail-first was aspirated by the cell of the sperm into the pipette injection, a mature oocyte which was matured was installed thru grabbing the pipette with the body of first polar at the position of six o’clock. The plan of oocyte at 3 o’clock position, the pipette of injection was introduce at 3 o’clock and oolemma rupture was achieved by small suction. The cell of sperm was given into the oocyte accompanied with thee minimal volume of medium and the pipette could be withdrawn cautiously, the procedure conducted in a dish of plastic microinjection including 10-μl droplets of (ferticult TM-HEPES, Belgium) buffered medium covered with mineral oil. After injection steps, oocytes were cultured and washed in micro-droplets wrapped with oil which was light paraffin. They are incubated at 37°C in an environment of PH of 7.2-7.4 5%CO2, and 5%O2. Next is embryo cleavage and fertilization, the oocytes were examined for fertilization next day after ICSI. Oocytes were deemed to be natural fertilized as 2 polar bodies were presented simultaneously accompanied with 2 clear observable pronuclei (two PN).

Intracytoplasmic sperm injection was carried out on all morphologically intact oocytes that have extruded the first polar body (metaphase II). In Intracytoplasmic sperm injection (ICSI) procedure, a single motile spermatozoon is selected and immobilized by pressing its tail between the 33 microneedle and the bottom of the dish. The sperm cell is then aspirated tail-first into the injection pipette, a mature oocyte is fixed by holding pipette with the first polar body at the 6 o’clock position. The plan of oocyte at 3 o’clock position, the injection pipette is introduce at 3 o’clock and rupture of the oolemma is ascertained by slight suction. the oocyte is delivered into the semen cell with minimal volume of medium and the pipette can be outgoing accurately, the steps executed in a plastic microinjection dish involving 10-μl droplets of (ferticult TM-HEPES, Belgium) buffered medium covered with mineral oil. in accordance with injection steps, oocytes cultured and rinsed in micro-droplets...
enveloped with paraffin oil. They sperm are brood at 37°C in an an weather of 5% O2, 5% CO2 and PH of 7.2-7.4. Subsequent is embryo cleavage and fertilization, the oocytes were investigated the next day for fertilization after ICSI. In addition, they are deemed oocytes to be normally fertilized when two polar bodies are presented together with two clearly visible pronuclei (two PN).

Fertilization is assessed sixteen–eighteen hours post the ICSI and the cleavage rate was tested two–three days after the retrieval of oocyte. Transformation of embryo was conducted after the retrieval of oocyte process on the fifth day at the phase of blastocyst. The subjects of the study were scheduled for embryotransfer (embryos were transferred by using trans-cervical catheter either fresh or freeze-dried embryos (Cook catheter Ob/Gyn, USA) to the uterus under abdominal ultrasound guidance). The pregnancy was assured by measuring the serum HCG concentration 12 days after the transfer process of embryo. The phase of Luteal was supplied by 50mg progesterone in oily injection IM and lasted till the detection of fetal heartbeat.

Main outcomes measures: In this study were duration of stimulation (days), numbers of (75 IU) gonadotropin ampules, estradiol levels on day of hCG, mature follicles numbers, endometrial thickness, numbers of oocytes retrieved, numbers of total embryos, number of embryo transfer, +ve pregnancy test, then u/s to confirm pregnancy and follow up until 12 weeks. Also measure Women’s serum samples for antithyroid antibodies.

Regarding the thyroid peroxidase antibody, as being <9 IU/ml and for thyroglobulin antibody as being <4 IU/ml, the normal values were accepted. The samples of Blood taken from venous blood from your arm IU/ml after collection of the whole blood allow the blood to clot by leaving it undisturbed at room temperature this usually taken 15-30 minute removed the clot by centrifuged at 1000-2000 x g for 10 minute in a refrigerated centrifuge the resulting supernatant is a serum then send to laboratory to measure value of antithyroid antibody

**Results**

A total of 81 patients were enrolled in the study. The parameters were summarized in the table (1):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Statistical N=81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>30.22±6.24</td>
</tr>
<tr>
<td></td>
<td>19-45</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>27.72±2.49</td>
</tr>
<tr>
<td></td>
<td>22-33</td>
</tr>
<tr>
<td>Duration of infertility (year)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>8.37±3.87</td>
</tr>
<tr>
<td></td>
<td>2-19</td>
</tr>
<tr>
<td>Basal FSH (IU/L)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>4.87±1.83</td>
</tr>
<tr>
<td></td>
<td>1.3-9.8</td>
</tr>
<tr>
<td>Basal LH (IU/L)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>3.40±1.70</td>
</tr>
<tr>
<td></td>
<td>0.6-9.1</td>
</tr>
<tr>
<td>TSH (u IU/L)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>1.93±.86</td>
</tr>
<tr>
<td></td>
<td>0.13-3.60</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>32.48±11.19</td>
</tr>
<tr>
<td></td>
<td>5.8-66.0</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
</tr>
<tr>
<td></td>
<td>26.94±8.19</td>
</tr>
<tr>
<td></td>
<td>11.2-54.0</td>
</tr>
<tr>
<td>Total Gn. Dose (IU)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>2206.48±766.83</td>
</tr>
<tr>
<td></td>
<td>1800.00</td>
</tr>
<tr>
<td></td>
<td>900-3900</td>
</tr>
</tbody>
</table>

Table (1): the means of the parameters included in the study.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>N=81</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of infertility</td>
<td>primary. Infertility</td>
<td>65</td>
<td>80.2%</td>
</tr>
<tr>
<td></td>
<td>secondary. Infertility</td>
<td>16</td>
<td>19.8%</td>
</tr>
<tr>
<td>Etiology of infertility</td>
<td>Male Factor</td>
<td>37</td>
<td>45.7%</td>
</tr>
<tr>
<td></td>
<td>Female Factor</td>
<td>12</td>
<td>14.8%</td>
</tr>
<tr>
<td></td>
<td>Unexplained</td>
<td>32</td>
<td>39.5%</td>
</tr>
<tr>
<td>Protocol</td>
<td>Agonist</td>
<td>69</td>
<td>85.2%</td>
</tr>
<tr>
<td></td>
<td>Antagonist</td>
<td>12</td>
<td>14.8%</td>
</tr>
<tr>
<td>Outcome Groups (pregnancy test)</td>
<td>Positive</td>
<td>47</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>34</td>
<td>42%</td>
</tr>
<tr>
<td>Fate of Pregnancy N=47</td>
<td>continue pregnancy until12weeks</td>
<td>14</td>
<td>29.7%</td>
</tr>
<tr>
<td></td>
<td>Abortion</td>
<td>33</td>
<td>70.3%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>22</td>
<td>27.2%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>59</td>
<td>72.8%</td>
</tr>
</tbody>
</table>
The number and percentage of the patients included in the study, according type of infertility, etiology of infertility, protocol, outcome, fate of pregnancy and state of antithyroid.

The patients were divided into two subgroups antithyroid positive groups(n=22) and antithyroid negative groups(n=59) (table 3): There is no significant difference between duration of infertility, BMI, and age between two groups (antithyroid positive and antithyroid negative group) (P-value >0.05).

There is significant difference in the days of stimulation (P-value 0.047), Fertilization rate (P-value 0.0047), No. of available embryos (P-value >0.003), No. of transfer embryos (P-value >0.05) between two groups.

Table (3): The general characteristic in antithyroid positive and antithyroid negative groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Antithyroid groups</th>
<th>POSITIVE N=22</th>
<th>NEGATIVE N=59</th>
<th>Sig. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (Mean±SD)</td>
<td>31.86±6.49</td>
<td>29.61±6.09</td>
<td>0.150</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²) (Mean±SD)</td>
<td>28.36±2.08</td>
<td>27.47±2.60</td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td>Duration of infertility (years) (Mean±SD)</td>
<td>8.91±4.29</td>
<td>8.17±3.72</td>
<td>0.448</td>
<td></td>
</tr>
<tr>
<td>Duration of Stimulation (days)</td>
<td>11.41±1.26</td>
<td>10.86±1.01</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>No. of retrieval oocytes</td>
<td>7.36±3.22</td>
<td>8.83±4.71</td>
<td>0.182</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>42.95±22.38</td>
<td>47.11±20.04</td>
<td>0.0042</td>
<td></td>
</tr>
<tr>
<td>No. of available embryos</td>
<td>2.73±1.03</td>
<td>3.46±0.92</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>No. of transfer embryos</td>
<td>2.36±1.05</td>
<td>3.34±0.90</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

There were no significant difference in basal hormones level (s.FSH, s.LH, s.TSH, s.prolactin and E2 at day of trigger) between antithyroid positive and antithyroid negative groups (table 4). and there was significant difference in total gonadotrophin dose and basal E2.

Statistical analysis appeared there were no essential difference regarding duration of stimulation, number of retrieval oocytes between two groups.

In the antithyroid positive group, the fertilization rate, the number of available embryo and the number of transfer embryo were dramatically lower than antithyroid negative group.
Table (4): The basal hormones levels in antithyroid positive and antithyroid negative groups.

<table>
<thead>
<tr>
<th>Antithyroid groups Parameters</th>
<th>POSITIVE N=22</th>
<th>MEDAIN</th>
<th>NEGATIVE N=59</th>
<th>MEDAIN</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>MEDAIN</td>
<td>Mean±SD</td>
<td>MEDAIN</td>
<td></td>
</tr>
<tr>
<td>Basal FSH(IU/ML)</td>
<td>5.28±2.16</td>
<td>5.1</td>
<td>4.72±1.69</td>
<td>4.8</td>
<td>0.218</td>
</tr>
<tr>
<td>Basal LH(IU/ML)</td>
<td>3.58±1.71</td>
<td>3.0</td>
<td>3.33±1.70</td>
<td>3.1</td>
<td>0.558</td>
</tr>
<tr>
<td>TSH(uIU/L)</td>
<td>1.87±1.01</td>
<td>1.7</td>
<td>1.96±0.80</td>
<td>2.0</td>
<td>0.678</td>
</tr>
<tr>
<td>E2</td>
<td>28.55±7.03*</td>
<td>27.0</td>
<td>33.95±12.11</td>
<td>32.0</td>
<td>0.016</td>
</tr>
<tr>
<td>Prolactin(ng/ml)</td>
<td>24.75±8.30</td>
<td>25.0</td>
<td>27.75±8.07</td>
<td>25.0</td>
<td>0.143</td>
</tr>
<tr>
<td>Total Gn. Dose(IU)</td>
<td>2636.36±827.53*</td>
<td>2700.0</td>
<td>2046.19±683.30</td>
<td>1800.0</td>
<td>0.002</td>
</tr>
<tr>
<td>E2 at day of HCG trigger(pg/ml)</td>
<td>1964.64±779.11</td>
<td>1999.0</td>
<td>2641.46±3695.15</td>
<td>2363.0</td>
<td>0.188</td>
</tr>
</tbody>
</table>

Regarding the outcome in patients in antithyroid positive groups and antithyroid negative groups, the antithyroid negative groups had higher rate of abortion compared to antithyroid positive groups (P value <0.001) while the continuity of pregnancy was higher in the antithyroid negative groups compared to antithyroid positive groups (P value <0.001) (figure 1).

In contrast to that there was no essential difference in the failure to get pregnancy between antithyroid positive groups and antithyroid negative groups (figure 1).

![Figure (1): The outcome in patients in antithyroid positive groups and antithyroid negative groups.](image)
Discussion

The present cross section study assessed the relationship between antithyroid antibody and pregnancy outcome intracytoplasmic sperm insemination (ICSI), hence a total of eighty-one Iraqi women were enrolled in this study of them 22 positive antithyroid antibody, 59 negative antithyroid antibody were undergone ICSI in Al-sader infertility center the group of the study contained only euthyroid female with no clinical manifested autoimmune illness.

It seems unlikely that antithyroid antibody (ATA) decreases the rate of pregnancy (no significant difference regarding failure to get pregnant among antithyroid positive groups and antithyroid negative groups). (p-value 0.493).

Hypothyroidism (subclinical define as upper normal level of TSH with elevated level of free T4) may be associated with negative results such as failure to conceive, pre-eclampsia, perinatal mortality, preterm birth, and miscarriage. Moreover, normal TSH, subclinical hypothyroidism, were reported until two prevent of pregnancies.10(11)

The limitations of the current research is the assessment of results of pregnancy after ICSI–embryo transmit cycle and moderately low quantity of patient because narrow time of follow up.

Confounding thyroid dysfunction was excluded in this study by including only women with normal TSH concentrations. Relationships between thyroid autoimmunity and unexplained infertility, miscarriage, recurrent miscarriage, preterm birth and maternal post-partu thyroiditis were reported independently from thyroid hormone concentrations(12).

In the study antithyroid positive group, the rate of fertilization, the available number of embryo and the quality of transfer embryo were dramatically lower than antithyroid negative group. The receptors thyroid hormone were labelled in human oocyte and can help in the trophoblastic differentiation and stimulation in the function of granulosacell (13). It was recommended that antithyroid antibodies have the responsibility for reducing fertility possible thru restricting the receptors(12). Therefore, the effect of the antibodies on the IVF cycles results were examined. despite the ATA existing is related with low result of IVF.

The antithyroid negative groups had higher rate of abortion compared to antithyroid positive groups (P value <0.001).

ATA presence is connected with the high risk of miscarriage in spontaneous pregnancy(14). Anyway, the rate of miscarriage is not higher in pregnancy of IVF attained in ATA-positive women compared with ATA-negative women in another metanalysis.

Studies found that the stimulation of ovarian might have suppressive impact on the immunity of humoral during the transfer of embryo despite the fact that the number is limited, the study have not negative effect of antithyroid antibodies on the rate of miscarriage in females considered by transfer of ICSI–embryo (15).

Alexander EK et al (2017) in infertile female, preconceptional TSH 2.5 mIU/L is not connected with contrary reproductive results; anyway, anti-TPO are connected with miscarriage high risk and reduced possibility of live-birth. This study is disagree with our study. (16)

K. Łukaszuk et al (2015) individuals diagnosed of anti-TPO antibodies regarding the rates of live birth, rates of pregnancy, implantation, fertilization revealed there was no significant differences also, no high danger regarding miscarriage after IVF-ET comparing with the negative for anti-thyroid antibodies compare with our result disagree(17)

Benaglia L et al (2013) the research showed that sick female diagnosed with anti-TPO antibodies regarding rates of live birth, rates of pregnancy implantation, fertilization there was no significant differences, moreover, regarding miscarriage, no high danger was found following IVF-ET comparing with the adverse for anti-thyroid antibodies this study disagree with our study. (18)

R. C. Smallridge et al (2013). Due to the unclear evidence, the study can’t suggest screening for the euthyroid treatment or thyroid autoantibodies for patients with positive thyroid autoantibodies at the pregnancy time.(19)
Prummel MF et al (2012) the antithyroid antibody existing is harmful for the results of pregnant after IVF-ET, therefore, duture research to examine suitable treatments to control function of the immune of ATA positive individuals to diagnosed enhance the result of IVF agree with our research.\(^{(20)}\)

Grtner, et al (1)(2009) Anti-thyroid antibodies, although it is not connected with thyroid dysfunction, are supposed to lead to a poor results of in vitro fertilization this agree with our study.\(^{(21)}\)

Limitations of the current research could be described as the pregnancies results assessment of using ICSI–embryo transfer cycles and relatively low number of patient because narrow time of follow up.

**Conclusions**

The antithyroid antibody’s presence is harmful for the results of pregnancy using ICSI-ET in euthyroid infertile women in the absence of other autoimmune disease. Hence we recommended further studies with estimated freeT4 in addition toTSH and large sample size and prolong time for follow up further studies should investigate suitable treatments to design immune function of ATA positive patients to improve IVF result.

**No conflicts of Interest**

**Source of Funding:** Self

**Ethical Clearance:** was taken from the scientific committee of the Iraqi Ministry of health

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The Effect of Conventional Method on the Length of Treatment Days for Children Underwent Circumcision at Poniran Khitan Centre Bengkulu

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Abstract

Background: Circumcision is a minor surgical procedure that is most widely performed in the world, namely a method of cutting all or part of the prepuce of the penis for specific indications and purposes.

Study Design: This study was used one-group pretest-posttest pre-experimental research design, with a total sample 15 respondents.

Results: Based on the results of the bivariate analysis using the Dependent T test, it was found that there was an effect conventional method with a value (p value = 0.000) on the on the length of days of care for circumcision wounds in children undergoing circumcision at the Poniran Khitan Centre Bengkulu.

Conclusion: The role of the Poniran Khitan Center will greatly assist in facilitating children in treating wounds who undergo circumcision

Keywords: Children, Circumcision, Conventional method

Introduction

Circumcision (circumcision) is the process of cutting the foreskin or prepuce of the penis by leaving the mucosa (inner layer of the skin) from the coronarious sulcus towards the head of the penis, which aims to prevent the buildup of smegma on the penis for social, religious or cultural reasons.

According to the United Nation of Acute Immuno Deviency Syndrome almost 30% of men are circumcised, and two out of three men are Muslim. The practice of circumcision is generally known in the Asian region, one of which is Indonesia. The implementation of circumcision can be influenced by several factors, including health, religion, sexuality and the application of social norms and the timing of circumcision in different countries and regions, depending on country and ethnicity.

Medically there is no age limit for circumcision. In the Indonesia World Health Organization, the most common age for circumcision is 5-12 years. The incidence of circumcision in each country varies according to religion, ethnicity, socio-economic status for medical, religious, social and cultural reasons. In 2013, it was found that 2.7 million people in 14 priority countries in eastern and southern Africa performed medical circumcision on men. Whereas in Indonesia 10.2 million perform circumcision. The prevalence of circumcision in the world is most prevalent in the world where the population is Muslim, namely 70%. In America 71.2%, Africa 44.7%, China 14%, Spain 6.6%, Australia 26.6%, India 13.5%, Japan9%, Afghanistan 99.8% and Thailand 23.4%.

Circumcision has many benefits, one of which reduces the risk of HIV transmission from women to men by 50-60% in Africa. The effectiveness of postcircumcision tissue wound repair is at the core of the problem that must be achieved in the current development of health science, so that the treatment of circumcision itself has attracted the attention of generations of health services in each country, including various technical strategies to improve and accelerate the healing time of...
circumcised patients.

The initial survey, which was conducted on July 29, 2019 at Poniran Khitan Center Bengkulu, obtained data in 2019 as many as 252 children. In 2018 there were 148 children, while in 2017 there were 163 children. Previously at the Poniran Khitan Center Bengkulu, a study was conducted to see the length of treatment days for children undergoing circumcision. Based on the above background, the researcher is interested in taking a research entitled The Effect of Conventional Methods on the Length of Care Days in Children Underwent Circumcision in Poniran Circumcision Center Bengkulu.

**Method and Method**

This research is a quantitative research with the type of pre-experimental research one-group pretest-posttest. This research conducted in June 2020 which was conducted at the Poniran Khitan Center, Bengkulu. The population in this study were children who underwent circumcision. The sampling technique in the study used non-probability sampling techniques, namely consecutive sampling. Sample selection is done by establishing inclusion criteria. Inclusion Criteria:

a. Children who underwent the clamping method who were willing to become respondents

b. Children who undergo circumcision do not experience interference with the visual and hearing system

This research data collection procedure starts from administrative procedures where getting a letter of passing the ethical test, processing a research permit to the Poniran Khitan Center Bengkulu. Furthermore, selecting potential respondents based on inclusion criteria, asking the child’s family’s willingness to become respondents and explaining the research objectives. Furthermore, the researcher asked the identity of the respondent, explained about the research on circumcision wound care and then observed it for 14 days of treatment.

**Result**

**Univariate Analysis**

The results of the univariate analysis of this study used to see the description of the frequency distribution of clients based on the conventional method before intervention and after the intervention in children who underwent circumcision at the Poniran Khitan Center Bengkulu:

<table>
<thead>
<tr>
<th>Table 1: Distribution of Respondents Based on Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Based on table 1, the frequency distribution of the age respondents children who underwent circumcision, Bengkulu, It is known that the age of the respondent is 7 years old as many as 10 respondents (66.7%), 8 years old as many as 5 respondents (33.3%).

**Bivariate Analysis**

The results of the bivariate analysis were used to see the effect of conventional methods on the treatment of circumcision wounds in children undergoing circumcision at Poniran Khitan Center Bengkulu. The results of these researchers themselves can be seen in the following table 2:

<table>
<thead>
<tr>
<th>Table 2: Distribution of Average circumcision wound care rates Respondents Before and After the Intervention in the conventional method Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Conventional Method</td>
</tr>
<tr>
<td>Pre Intervention</td>
</tr>
<tr>
<td>Post Intervention</td>
</tr>
</tbody>
</table>

Based on table 3, the results of the analysis of the average pre-intervention wound care result was 17.53 with a standard deviation of 1.807. After giving the treatment intervention, it was obtained an average of
8.93 with a standard deviation of 1.163. The results of the statistical test with a p value of 0.000, it is concluded that there is a significant influence between conventional methods before and after giving wound care interventions to children undergoing circumcision at the Poniran Khitan Center Bengkulu.

Discussion

Univariate Analysis

Based on table 1, it is known that the age of the respondents. The results of this study are one of the manifestations that most of the children who undergo circumcision are 7 years old.

According to Wong (2008), the characteristics of school-age children can be seen from several aspects, including in terms of independence and spiritual development. School-age children are able to do coping effectively and are able to cooperate with health workers. Although school-age children have an independent nature that is able to work with health workers, there are several factors that can make him anxious.

According to Hockbenberry (2015), States that the younger the child is, the more difficult it is to adjust to a new environment, in this case an unfamiliar environment and action procedures. According to researchers, the age of the child undergoing circumcision is the sooner the better. This is because it makes it easier to heal wounds.

Bivariate Analysis

Based on the results of the dependent t statistic test, it is known that conventional wound care before the intervention was 17.53 with a standard deviation of 1.807. After giving the intervention, the average circumcision wound care was 8.93 with a standard deviation of 1.163. The statistical test results obtained p value of 0.000, so it can be concluded that there is a significant influence between wound care before and after giving intervention to children undergoing circumcision at Poniran Khitan Center Bengkulu.

In line with Maizels’ (2019) research on “Outcome of circumcision for newborns with penoscrotal web: oblique skin incision followed by penis shaft skin physical therapy shows success” results obtained from 828 boys who came for circumcision, 652 (79%) were registered because they were suitable for circumcision: 355 (43%) in the normal group and 297 (36%) in the web group. The remaining 176 (21%) were excluded because they had penile anomalies: buried penis (125), chordee (40), and hypospadias (11). Follow-up was carried out for 6 months. In the web group, follow-up data were obtained for 263 of 297 (89%) cases, with 261 of 263 (99%) showing success, and in the normal group, follow-up data were obtained for 327 of 355 (92%) cases, with all 327 (100%) indicated success. Two cases in the web group (0.7%) failed to undergo surgical reconstruction.

Another study by Alpert (2018) on Combination treatment for cicatrix after neonatal circumcision: An office-based solution to a challenging problem with the results of research namely the formation of a cicatrix, which can form after neonatal circumcision to trap the glans penis, presents a therapeutic challenge. Previous studies in the literature have described the use of topical steroid creams or stretching of scar tissue with instruments but not a combination of the two modalities. Based on our experience, monotherapy has caused significant relapses and/or requires further treatment. We present our successful experience combining cicatrix stretching with hemostats using local anesthesia in the office followed by topical steroids for several weeks with a minority of patients requiring additional therapy.

According to researchers, the process of treating a circumcision wound will be fast if it is according to the procedure between the child who is undergoing circumcision and the treatment so that the wound will recover quickly. In accordance with the theory, the length of wound care days is 14 days from the time the wound is opened, so in this study the length of wound care is in accordance with the days of wound healing.

Conclusion

The frequency distribution of child respondents who underwent circumcision with conventional methods was 7 years old as many as 10 respondents (66.7%) and 5 respondents (33.3%) aged 8 years.

The average value of wound care using conventional methods, namely the average value before that is 17.53 and the average value after intervention is 8.93.
There is an effect of conventional methods on the length of days of care for children undergoing circumcision at the Poniran Khitan Center Bengkulu.

Conflict of Interest: The authors declare that there is no conflict of interest.

Source of Funds: This research is a Beginner Lecturer Research with funding sources from Ristekdikti grants.

Ethical Clearance: Health Research Ethics Committee, Health Polytechnic of Health Ministry Bengkulu

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Neutrophil Lymphocyte Ratio and Acid-Fast Bacilli in Tuberculosis

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Abstract

Background: Tuberculosis (TB) is one of the top 10 causes of death in the world and the most common cause of death with single infectious agent. The neutrophil-lymphocyte ratio is a cheap and fast marker of inflammation that has been widely studied in TB. The aim of this study was to investigate Neutrophil-lymphocyte ratio (NLR) in TB with positive Acid-Fast Bacilli (AFB) and negative AFB. Methods: This was a retrospective study that included TB patients data from the medical records in two hospitals in Palu, Indonesia. A total of 150 TB patients were involved in this study consisting with positive and negative AFB TB suspect. Neutrophil, lymphocyte and NLR data were compared with the Mann-Whitney test. Results: The study shows a significant difference of neutrophils and lymphocytes percentage between positive AFB TB patients (80 subjects, 53.3%) and negative AFB TB patients (70 subjects, 46.7%) with (p < 0.001) as well as NLR (p < 0.001). The percentage of Neutrophils was significantly higher in positive AFB TB compared to negative AFB tuberculosis (81.18 ± 8.52 vs. 55.02 ± 9.80), lymphocyte percentage were found to be significantly lower in positive AFB TB compared with negative AFB TB (12.72 ± 7.51 vs 28.69 ± 12.01). Additionally, NLR were significantly higher in positive AFB TB compared with negative AFB TB (10.20 ± 9.53 vs 2.47 ± 1.56). Conclusion: There is a significant increase in the number of neutrophils, a decrease in lymphocytes and an increase in NLR among positive AFB TB compared with negative AFB TB.

Keywords: Tuberculosis, Acid-Fast Bacilli, Neutrophil, Lymphocyte, Neutrophil Lymphocyte Ratio

Introduction

Tuberculosis (TB) is one of the top 10 causes of death in the world and the most common cause of death with single infectious agent more than human immunodeficiency virus (HIV). Millions of people suffer from TB every year. In 2017, TB was estimated to cause 1.3 million deaths in HIV-negative patients and 300,000 deaths in HIV-positive patients (1). The activation progress of TB is a combination of bacterial virulence factors and host immune system factors. Recently advanced knowledge of immunology in TB provides an overview of abnormalities in hematology (2).

One of the criteria for TB is by examining acid-fast bacilli (AFB). High quality and sufficiency of sputum sampling is very important to obtain good result. Therefore, patients need to understand good sputum sampling. Good sputum is sputum containing material from the lung when productive coughing and not from the nasopharynx only. One of methods to determine quality of sputum sample is to assess sputum leukocyte...
levels \(^{(3)}\), but this is time consuming and costly. Despite its lack of sensitivity compared to sputum culture, AFB is still the mainstay of diagnosis of tuberculosis with positive AFB among the pulmonary tuberculosis suspect patients in many regions \(^{(4)}\). The use of AFB examination alone without confirmation by culture, the quality is needed to fully assured in its use \(^{(5)}\).

Various factors can influence the results of AFB examination, including the quality of the sample and the competency of the examiner so that the AFB examination has its own constraints in determining the diagnosis of TB patients. The number of bacteria in sputum may be related to bacterial virulence, higher virulence bacteria have better endurance and replicate in macrophages or neutrophils faster than low virulence bacteria \(^{(6, 7)}\). Cellular immune responses especially those produced by T lymphocytes play an important role in controlling the replication of *Mycobacterium tuberculosis* (MTB) as well as neutrophils play a role in innate immunity to MTB. Lymphopenia, neutrophilia and monocytosis are the most common hematological abnormalities in TB \(^{(8, 9)}\).

The neutrophil-lymphocyte ratio (NLR) is an inflammatory marker that can be used to estimate various clinical conditions including cancer and coronary heart disease and also shows differences based on the type of infection. In addition, NLR is also useful to predict and detect inflammatory conditions and infections. NLR of miliary TB patients at the time of hospital admission can be used as a marker of mortality and the risk of acute respiratory distress syndrome (ARDS) \(^{(10-12)}\). Other studies have also shown that NLR is found to be increased in patients newly diagnosed with TB and NLR can be used as an inflammatory marker that can help management of TB patients and to determine the severity of TB disease \(^{(2, 9)}\). AFB and NLR obtain from leukocyte differential count are widely used and more affordable in Indonesia specially in rural areas. The objective of this study was to see differences of NLR in TB with positive AFB and negative AFB.

**Method**

This research is a retrospective study utilizing data from medical record of TB patients in two hospitals in Palu City, Indonesia (Undata Hospital in 2016 and Anutapura Hospital in 2017). TB patients were included and examined whether they had carried out AFB examinations, then divided into 2 groups based on examination AFB, positive AFB and negative AFB. Hematological examination data of the two groups was taken to determine the levels of neutrophils and lymphocytes. The patients were excluded from the study if AFB and hematologic examination were unavailable. Generally these patients have undergone TB therapy. 88 samples were taken from the Undata Hospital and 62 samples were taken from Anutapura Hospital, the total number of samples was 150 TB patients.

Data distribution was tested by the Kolmogorov-Smirnov Test. Data with normal distribution has a value of \(p > 0.05\). The Kolmogorov Smirnov test show that the distribution of neutrophils, lymphocytes and NLR data is not normal. The statistical test used to compare the two groups was the Mann-Whitney test because of abnormal data distribution.

**Result**

The total number of samples was 150 TB patients consisting of 80 smear of positive AFB TB and 70 negative AFB TB. The age of positive AFB TB patients was 43.20 ± 13.16 years with a range of 21-72 years, the age of patients with negative AFB TB was 41.32 ± 14.07 with a range of 17 - 63 years. Male in the positive AFB TB group was 60% and 40% female, in the negative AFB TB group male was 56% and female 34% (table 1).

The Mann-Whitney test showed a significant differences of neutrophils and lymphocytes percentage between positive AFB TB patients and negative AFB TB \((p < 0.001)\), as well as NLR \((p < 0.001)\). Neutrophils percentage was significantly higher in positive AFB TB compared with negative AFB TB \((81.18 ± 8.52 vs. 55.02 ± 9.80)\), lymphocytes percentage were significantly lower in positive AFB TB compared with negative AFB TB \((12.72 ± 7.51 vs 28.69 ± 12.01)\). NLR were significantly higher in positive AFB TB compared with negative AFB TB \((10.20 ± 9.53 vs 2.47 ± 1.56;\) table 2).
Table 1. Sample characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive AFB (n = 80)</th>
<th>Negative AFB (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (year)</td>
<td>43.20 ± 13.16</td>
<td>41.32 ± 14.07</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>48/32</td>
<td>39/31</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>81.18 ± 8.52</td>
<td>55.02 ± 9.80</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>12.72 ± 7.51</td>
<td>28.69 ± 12.01</td>
</tr>
<tr>
<td>NLR</td>
<td>10.20 ± 9.53</td>
<td>2.47 ± 1.56</td>
</tr>
</tbody>
</table>

AFB = Acid-Fast Bacilli; NLR = Neutrophil Lymphocyte Ratio

Table 2. Comparison of Neutrophils, Lymphocytes and NLR between positive AFB TB and negative AFB TB

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive AFB (n = 80)</th>
<th>Negative AFB (n = 70)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil (%)</td>
<td>81.18 ± 8.52</td>
<td>55.02 ± 9.80</td>
<td>0.000**</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>12.72 ± 7.51</td>
<td>28.69 ± 12.01</td>
<td>0.000**</td>
</tr>
<tr>
<td>NLR</td>
<td>10.20 ± 9.53</td>
<td>2.47 ± 1.56</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

*Significant < 0.05; **Significant < 0.001

Discussion

Tuberculosis is an infectious disease which the progression and the outcome depend on the immune reactivity of the host (13). The immune system helps control the proliferation of MTB. Reactivation of latent TB occurs in individuals who experience a decrease in the immune system (14, 15).

Inflammation plays an important role in the pulmonary TB pathogenesis as in protection and pathology. Yaranal et al showed that hematologic abnormalities were often found in severe TB cases. Hemoglobin levels, platelet counts, lymphocytes and neutrophils counts change in the chronic inflammatory process and return to normal with effective therapy. These parameters can be used as indicators to assess treatment (2, 13, 16, 17). In the early stages of the disease proinflammatory cytokines and chemokines released by various cells, induce migration of immune cells to infected areas, form granulomas and initiate host response protection. The cellular population increases in response, involving alveolar macrophages, dendritic cells, neutrophils, NK cells, epithelial cells and other cells (13).

Jadoon et al. showed that decreased lymphocyte levels and increased neutrophil levels were associated with the severity of TB disease (18). The immune response often has a certain pattern, which were an increase in the number of neutrophils and a decrease in the number of lymphocyte cells. When infection persists, neutrophil production increases and the possibility is not apoptosis. Neutrophil apoptosis in sepsis is beneficial, whereas lymphocyte apoptosis provides a loss (19). Polymorphonuclear cells (PMN) are cells that play a role in phagocytosis and are the first line of defense against microbes. The highest PMN in the blood is neutrophils. In TB infection, PMN will be attracted to infected areas along with interleukin 8. In patients with active pulmonary TB, PMN becomes the dominant cell found in the lungs (20).
The role of neutrophils in TB is still debatable. Several studies demonstrated the involvement of neutrophils in granuloma formation, initiation of T cell responses, amplification of antimicrobial activity of macrophages, secretion of antimicrobial peptides and killing of tuberculosis. This implies that neutrophils have many different roles in each phase of TB, mediating protection in the onset phase of infection and pathology induction in the later phase of TB. There are some mechanisms of neutrophil induction of pathology in TB, neutrophils were suggested to play the role of trojan horse by hiding MTB from active macrophages. With increasing neutrophils and precursors, neutrophils are likely to suppress T cell responses (21). Neutrophils help early CD4 activation of T cells by working with dendritic cells in mice infected with virulent MTB. Neutrophils are predominant cells infected with MTB in TB patients and these bacteria replicate intracellular (7, 22). Research by Xu et al. showed that the percentage of regulatory T cells increased in patients with negative AFB compared with patients with positive AFB. Regulatory T cells are thought to play a role in preventing inflammation which causes damage to host tissues in TB (23, 24).

Calculations of NLR are easily obtained from the results of routine laboratory examinations, and can be done in many hospitals. NLR is also easier to obtain and cheaper than other inflammatory markers such as C-reactive protein and procalcitonin (2, 25). The role of NLR as an inflammatory marker in TB has been widely investigated (26-30). This study showed that higher neutrophils percentage and lower lymphocyte percentage were found in positive AFB TB compared with negative AFB TB. Both of these contribute to the increased value of NLR in positive AFB TB. Tuberculosis with positive AFB is considered to have more bacterial load in sputum and could be more virulent than negative AFB TB. The NLR in this study can illustrate the level of inflammation of TB infection but there are findings that shows high numbers of neutrophils play a role in the replication of TB bacilli.

The limitations of the study include the absence of the duration or type of TB patients treatment, the severity of clinical symptoms and radiological features. Further research is needed to analysis NLR in TB patients based on therapy, relapse, HIV co-infection, nutritional status, molecular test and TB drug resistance. Research with more patients is needed to determine the cut-off value of NLR in positive AFB and negative AFB TB.

Conclusion

This study indicates that in positive AFB TB has an increase in neutrophils percentage, a decrease in lymphocytes percentage and an increase in NLR compared with negative AFB TB.

Conflict of Interest: The authors declare that they have no conflict of interest.

Acknowledgments: We thanks to the contribution of the management of Undata Hospital, Palu, Indonesia and Anutapura Hospital, Palu, Indonesia. We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

Funding : None

Ethics Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Faculty of Medicine, Tadulako University, Palu, Indonesia.

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The Extent of the Need for Teaching Forensic Sciences for Law Students in Jordanian Universities

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Abstract

This article examines the actual need for teaching forensic sciences to Bachelor of Law students in Jordanian universities, where the research extrapolated the study plans for the specialization of law in the faculties of law in Jordanian universities, which amount to 20 faculties, to know the faculties that teach forensic sciences. It was found that only one faculty offered forensic sciences as an elective requirement, and that 13 faculties included among their study plans the course of forensic medicine as an elective requirement.

Also, a questionnaire was designed and distributed to a random sample of 120 lawyers in Jordan. The questionnaire contained 9 multiple-choice questions, the most important results are that only 39% of the sample studied one of forensic sciences subjects at the bachelor’s level, and 36% of them know the work of forensic experts, and 92% support that the forensic sciences to be as a compulsory requirement course for law students.

The article discussed the results of the questionnaire in a scientific discussion and concluded the necessity to design a subject specialized in forensic sciences for students of law, provided that it is a compulsory requirement because of its great importance in the work.

Keywords: Forensic sciences, Education, Justice, Law study.

Introduction

Forensic sciences are considered one of the modern sciences of distinctive importance due to the important services it provides to justice through the use of scientific means to uncover crimes. It’s distinguished by the fact that it uses various modern means and techniques to uncover crime.

By forensic sciences, we mean those sciences that assist in uncovering crimes, clarifying their mysteries, determining the methods of their commission, and tracing the perpetrator and tracing his traces through physical evidence¹. These sciences play an important role in revealing the truth, especially in criminal cases, and to the extent that these sciences are advanced, the results reached by the judiciary are identical to reality, thus achieving reassurance in judgments².

It is noticeable that forensic sciences has developed remarkably in the modern era, expanding its scope to include several branches of the sciences American Academy of Forensic Sciences, for example, includes the following specific areas of expertise: pathology and biology, toxicology, criminalistics, questioned documents, forensic odontology, anthropology, jurisprudence, psychiatry, and a general section. Other sections are developing in such fields as engineering, geology, and microscopy³.

There is no doubt that the use of Forensic sciences at present time has become very important, as most courts resort to these sciences to reach the certainty of truth, and this is what leads us to raise the topic of this article,
which is, is it now necessary to teach Forensic sciences for law students?

The article used two methods to arrive at results. Initially, a survey study of the study plans for the Bachelor of Laws program was conducted for all 20 Jordanian universities, to know the reality of teaching Forensic sciences in law faculties. After knowing the results of this study, I designed a questionnaire that contained 9 multiple-choice questions, with the aim of the reality of lawyers knowledge of forensic sciences and the extent to which they needed to study forensic sciences or not.

A random sample of 120 Jordanian lawyers was selected, all of whom were graduates of Jordanian law schools, whose experience in the profession ranged from one to thirty years.

The questionnaire contained the following questions:

- Have you studied forensic sciences or one of its branches in the Bachelor?

- Do you have interests and following new information in forensic sciences?

- When you want to know any information in the forensic sciences, your method for that is:

- Do you have the ability to discuss forensic experts with scientific information?

- Have you ever asked the court to resort to forensic evidence to deny the accusation?

- What are the most important cases in which you think forensic sciences have a role?

- Have you ever seen how forensic experts work?

- What kind of evidence have you dealt with the most in your cases?

- Do you think it is necessary to study forensic sciences as a compulsory course at the undergraduate level?

**Result**

The study plans for all law faculties in Jordanian universities (government and private), totaling 20 faculties, were reviewed in each of the following universities: University of Jordan, Yarmouk University, Mutah University, Al Al-Bayt University, Al-Balqa Applied University, Al-Hussein Bin Talal University, Applied Science Private University, Al-Ahliyya Amman University, University of Petra, Israa University, Al-Zaytoonah University, Middle East University, University of Islamic Sciences, Philadelphia University, University of Jerash, Irbid National University, Jadara University, Ajloun National University, Zarqa Private University, and Amman Arab University.

It turns out that one university, which is Applied Science Private University, teaches criminal science as an elective course for law students. According to the description announced by the faculty, “This course is devoted to the study of one of the auxiliary criminal sciences chosen by the teacher: Criminology, penology, criminal policy, judicial psychology, a criminal investigation, criminal medicine, etc”.

It was also found that thirteen universities teach (forensic medicine) as an elective course for students of the Faculty of Law. These universities are: University of Jordan, Yarmouk University, Mutah University, Al Al-Bayt University, Al-Balqa Applied University, Al-Hussein Bin Talal University, University of Petra, Al-Isra University, Al-Zaytoonah University, Jerash University, Irbid National University, Ajloun National University, and Amman Arab University. The description of the course was the same in all these universities, which “This course aims to study the definition, means of requesting help from forensic medical doctors in judiciary proceedings and investigations including medical reports, inspection of dead bodies, their autopsy and reasons of death”.

As for the results of the questionnaire, it was as follows:

1- (39%) of the study sample studied one of the forensic sciences subjects at the bachelor’s level, compared to (61%) of them did not study any of its branches.

2- (80%) of the study sample have an interest in forensic sciences and follow up on new information.

3- (55%) of the study sample obtains their information in forensic sciences from the Internet, and
(37%) of them through asking experts, while (8%) of them resort to specialized books and journals.

4- (58%) of the study sample believes that they can discuss scientific information with forensic experts.

5- (49%) of the study sample had previously asked the court to resort to scientific evidence to deny the accusation from the accused.

6- (52%) of the study sample believed that the cases that most need resort to forensic sciences are those related to murder and abuse, while (46%) of them believe that are the cases related to sexual crimes, and (2%) believe that are cases related to property crimes.

7- Only (36%) of respondents know how forensic experts work.

8- The experience of Detecting Forgery And Counterfeiting constituted the most scientific expertise that the sample members dealt with (65 people), followed by the experience of traffic accidents (22 people), then the experience of the genetic fingerprint (14 people), and then the other experiences, and the experience of weapons came as the least type of experience dealing with Frequently (one person from the sample).

9- (92%) of the study sample believed that it is necessary to study forensic sciences as a compulsory course at the bachelor’s level, compared to (8%) of them who do not believe it is necessary.

Discussion

It seems that the study plans for the Bachelor of Laws program at Jordanian universities are still traditional so that still contain legal courses explain the texts of law, meaning that the basis for studying law in these universities stems from the legal text only, so these plans lack many of courses that helps law student to practice his profession. Therefore, it is important to include assistive courses that develop student’s skills, not only in interpreting and analyzing legal texts but also in linking all sciences with the science of law.

There is a clear neglect of forensic sciences during the study of law, and even with a number of faculties that teach forensic medicine as an elective requirement, this is not sufficient, as forensic medicine is only a branch of forensic sciences. In connection with the results of the questionnaire, there are branches of forensic sciences that are more used than forensic medicine, so the student must be familiar with them.

There is no doubt that there is recognition of the existence of a large gap between the scientific and legal disciplines, on the other hand there is recognition of the need to bridge this gap. As the legal experts realize the importance of resorting to scientific disciplines to solve their cases, it is evident from the results of the questionnaire that there is a desire of lawyers for the course of forensic sciences to be a compulsory course during their studies due to the importance they have seen on the ground.

Conclusion

Nothing can establish the foundations of justice in societies as far as revealing the truth that has become within reach by the development of science. From here appears the importance of studying forensic sciences for law students, a course that is lacking in our Jordanian university. With the awareness of those working in the legal field of this, it has become necessary to design a special curriculum for forensic sciences for law students, and we mean by a special curriculum that the presentation of information is consistent with the legal mentality, and this is what many writers who have written books in forensic medicine have directed especially for law students (for example See: Batrawy, 2004).

From this standpoint, we call on law faculties in Jordanian universities to amend their study plans to include at least a compulsory course in forensic sciences aim to introducing law students to modern scientific evidences.

Acknowledgments: The authors would like to thanks Applied Science Private University and Petra University/ Jordan.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Ethical clearance was not necessary as it was an Educational legal study which included only collection of data.
References


Does Husband/Partner Matter in Reduce Women’s Risk of Worries?: Study of Psychosocial Burden of COVID-19 in Indonesia

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Abstract

Several previous studies have found that women are more susceptible to psychosocial burden than men. The study was aimed at analyzing the influence of having a husband/partner on the psychosocial burden of COVID-19 among women in Indonesia. The study involved 5,061 women collected through a rapid online survey. The psychosocial burden was reviewed based on worries level on 5 aspects of daily life, namely economic, religious, educational, employment, and social aspects. Four independent variables were included in the analysis, including husband/partner ownership, age group, education level, and employment status. A multivariate test using the binary logistic regression was done at the final stage. The results show that women who have a husband/partner have a risk of 1.377 times compared to women who do not have husbands/partners to experience worries. Women in the 40-49 age group had 0.630 times the risk of women with ≤19 age groups to experience worries. Meanwhile, women with the ≥50 age groups had a 0.327 times risk than women with the ≤19 age groups for experiencing worries. Women with higher education have a risk of 0.610 times compared to women with secondary education for experiencing worries. It could be concluded that having a soulmate is a risk factor for women to experience the psychosocial burden of COVID-19 in Indonesia. The analysis also found that younger age and lower education were risk factors for women to experience the psychosocial burden of COVID-19 in Indonesia.

Keywords: women’s health, psychosocial burden, mental health, online survey, COVID-19.

Introduction

It is estimated that the decline in the number of COVID-19 incidents at the global level is far from over. Data from www.worldometers.info/coronavirus/ accessed on 11 August 2020, in the last 7 days, show that the global average rate of increase in new cases of COVID-19 has reached approximately 200,000 cases/day. In addition to the high daily average number of cases, at the global level, the average death rate due to COVID-19 in the last 7 days has reached approximately 6,000 deaths/day.

The situation is more or less the same in Indonesia. Based on the official website of the Indonesian government, www.covid19.go.id, which was accessed on 11 August 2020, the average number of new cases of COVID-19 in the last 7 days has reached approximately 2,000 cases/day. The average death rate due to COVID-19 in the last 7 days has reached approximately 70 deaths/day.

The Indonesian government has made various efforts to reduce the rate of spread of COVID-19. One of
the strategies undertaken by the Indonesian government is to issue the Minister of Health Regulation Number 9/2020 concerning Guidelines for Large-Scale Social Restrictions in the Context of Accelerating Handling of Corona Virus Disease 2019. This regulation emphasizes the existence of restrictions on community movement. The implication of this regulation is the closure of several locations, including educational institutions (schools), offices, tourist attractions, and so on.

The Minister of Health Regulation Number 9/2020 was strengthened by the existence of Presidential Instruction Number 6/2020 concerning Increasing Discipline and Law Enforcement of Health Protocols in the Prevention and Control of COVID-19. This Presidential Instruction ensures that there is a clear legal umbrella for the government to enforce discipline in the community so that it complies with existing health protocols, namely using masks, applying physical distancing, diligently washing hands.

Both regulations have an impact on various aspects of life in society, including religious, economic, social, and educational aspects. The phenomenon of the impact of this regulation can be seen from the existence of several reports in the mass media, including the existence of several people who work even though in their territory it has been forbidden to work\textsuperscript{1,2}. The duration of implementing social restrictions has been quite long. This condition triggers boredom so that there is a tendency for violations to occur\textsuperscript{3}.

In addition to the potential for violations to occur during the period of limitation, people also have the potential to experience depression and worries\textsuperscript{4}. The existence of hoax information also has a role in the occurrence of anxiety, so that this worsens the situation and conditions in society\textsuperscript{5–7}. This hoax news was then added to by the phenomenon of the conspiracy theory echoed by the leaders of the superpowers\textsuperscript{8}.

Worry is a psychosocial burden. The public health problem that needs attention. World Health Organization formulates the definition of healthy, one of which also includes mental dimension\textsuperscript{9}. Given the situations and conditions above, it is necessary to pay more attention to see the conditions of the psychosocial burden during the COVID-19 pandemic.

Several previous studies have found that women are more affected psychosocial under various uncertain stresses such as the current COVID-19 pandemic condition\textsuperscript{10–12}. Women are considered more vulnerable because of several conditions, including the incidence of female workers experiencing a decrease in productivity compared to male workers when working from home, besides that women are also burdened with domestic affairs in the household\textsuperscript{13–15}. The study was aimed at analyzing the influence of husband/partner ownership on the psychosocial burden of COVID-19 among women in Indonesia.

Materials and Methods

The study uses a rapid online survey to collect data about the worries felt by women in Indonesia. Data were collected for 8 days (June 6-13, 2020), until 5,061 women were obtained. In this study, the psychosocial burden was measured based on the worries level. The worries level was a self-assessment of anxiety in 5 aspects of daily life, namely economic, religious, educational, employment, and social aspects. Each aspect was assessed based on the choice of answers using a Likert scale. The worries level composite variable was composed of scores from 5 aspects measured. The total score was divided into 2 classes of the same size, which are not worries and worries. Four independent variables were included in the analysis, including husband/partner ownership, age group, education level, and employment status.

At the initial stage, a bivariate test with chi-square was carried out to see the relationship between the husband/partner ownership variable and other variables. In the final stage, a multivariate test using binary logistic regression was carried out to determine the risk factors for the psychosocial burden of COVID-19 among women in Indonesia. SPSS 22 software was used in all statistical credentials.

Results and Discussion

Table 1 is a display of bivariate analysis between husband/partner ownership and all variables involved in the analysis. It can be seen that in both categories of husband/partner ownership is dominated by women who experience worries. Meanwhile, based on the age group, women who don’t have husband/partner dominated by the 20-29 age group, and women who have husbands/
partners dominated by the 30-39 age group.

Based on the education level, women who don’t have a husband/partner dominated by secondary education, and women who have husbands/partners dominated by higher education. Furthermore, in the base on employment status, it can be seen that the two categories of husband/partner ownership are dominated by employed women.

### Table 1. Descriptive statistics of respondents (n=5,601)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Husband/partner</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Don’t have</td>
<td>Have</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Worries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· No</td>
<td>326</td>
<td>11.5%</td>
</tr>
<tr>
<td>· Yes</td>
<td>2516</td>
<td>88.5%</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· ≤ 19</td>
<td>577</td>
<td>20.3%</td>
</tr>
<tr>
<td>· 20-29</td>
<td>1924</td>
<td>67.7%</td>
</tr>
<tr>
<td>· 30-39</td>
<td>198</td>
<td>7.0%</td>
</tr>
<tr>
<td>· 40-49</td>
<td>59</td>
<td>2.1%</td>
</tr>
<tr>
<td>· ≥ 50</td>
<td>84</td>
<td>3.0%</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Secondary</td>
<td>1808</td>
<td>63.6%</td>
</tr>
<tr>
<td>· Higher</td>
<td>1034</td>
<td>36.4%</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Unemployed</td>
<td>1160</td>
<td>40.8%</td>
</tr>
<tr>
<td>· Employed</td>
<td>1682</td>
<td>59.2%</td>
</tr>
</tbody>
</table>

Note: ‘p<0.05; ‘’p<0.01; ‘’’p<0.001.

Table 2 displays the results of the binary logistics regression of the psychosocial burden on the COVID-19 among women in Indonesia. Based on the ownership of a husband/partner, women who have a husband/partner have a risk of 1.377 times compared to women who don’t have a husband/partner to experience worries (OR 1.377; 95% CI 1.096-1.730). The results of this analysis inform that having a husband/partner is a risk factor for women to experience the psychosocial burden of COVID-19 in Indonesia.

Having a husband/partner should be a place to share worries, but the results of the analysis show contradictory findings. Having a husband/partner increases the risk for women to experience the psychosocial burden of COVID-19. This condition is possible because of the increased worries as a result of the increasing number of family members. For a single woman, worry is only for herself. This condition is different for women who have a husband/partner, the burden of worries increases on the number to be worried about, not only husband/partner...
but also children. The findings in this study contradict several studies conducted in the United State regarding marital status. The studies inform that unmarried patients, including those who are divorced, separated, widowed, or never married, have an increased rate of adverse cardiovascular events when compared to their married counterparts.16–18

Table 2. The result of binary logistic regression of the psychosocial burden of the COVID-19 among women in Indonesia, in 2020 (n=5,601)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Worries</th>
<th>Sig</th>
<th>OR</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Husband/partner: Don’t have</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Husband/partner: Have</td>
<td>**0.006</td>
<td>1.377</td>
<td>1.096</td>
<td>1.730</td>
<td></td>
</tr>
<tr>
<td>Age groups: ≤ 19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age groups: 20-29</td>
<td>0.291</td>
<td>1.190</td>
<td>0.862</td>
<td>1.643</td>
<td></td>
</tr>
<tr>
<td>Age groups: 30-39</td>
<td>0.584</td>
<td>0.892</td>
<td>0.593</td>
<td>10.342</td>
<td></td>
</tr>
<tr>
<td>Age groups: 40-49</td>
<td>*0.034</td>
<td>0.630</td>
<td>0.411</td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>Age groups: ≥ 50</td>
<td>***&lt;0.001</td>
<td>0.327</td>
<td>0.214</td>
<td>0.500</td>
<td></td>
</tr>
<tr>
<td>Education: Secondary</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Education: Higher</td>
<td>***&lt;0.001</td>
<td>0.610</td>
<td>0.491</td>
<td>0.758</td>
<td></td>
</tr>
<tr>
<td>Employment status: Unemployed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Employment status: Employed</td>
<td>0.066</td>
<td>1.200</td>
<td>0.988</td>
<td>1.456</td>
<td></td>
</tr>
</tbody>
</table>

Note: ’p<0.05; “p<0.01; “”p<0.001.

Based on the age groups, women with the 40-49 age groups had 0.630 times the risk of women with ≤19 age groups for experiencing worries (OR 0.630; 95% CI 0.411-0.965). Meanwhile, women with ≥50 age groups had a 0.327 times risk than women with ≤19 age groups for experiencing worries (OR 0.327; 95% CI 0.214-0.500). This information indicates that the younger the more the risk is for women to experience the psychosocial burden of COVID-19 in Indonesia.

Old age is synonymous with a more complete experience in dealing with the ups and downs of life. Senior age is considered to have a more complete coping mechanism. This condition is considered to be able to minimize worries that occur.19–21

Furthermore, women with higher education had a risk of 0.610 times compared to women with secondary education for experiencing worries (OR 0.610; 95% CI 0.491-0.758). These findings inform that better education reduces the risk for women to experience the psychosocial burden of COVID-19 in Indonesia.

Having a better education goes hand in hand with a better understanding of COVID-19. This condition minimizes the occurrence of worries because of the increased ability to recognize risks and preventive behavior so that they can anticipate more readily. Including the impact of psychosocial burden due to the quarantine process.12,22,23 In general, education was reported in several studies as a positive determinant of
program performance in health24–26. Meanwhile, the low level of education is a barrier to various performance in the health sector to achieve better results27,28.

Conclusions

Based on the results and discussion it could be concluded that having a husband/partner is a risk factor for women to experience the psychosocial burden of COVID-19 in Indonesia. The analysis also found that younger age and lower education are risk factors for women to experience the psychosocial burden of COVID-19 in Indonesia.

Source of Funding: Self-funding

Conflict of Interests: Nil

Ethical Clearance: This study has received ethical approval from the National Ethics Commission (No: RK.05/KEPK/STIK/V/2020). The respondents’ identities have all been deleted from the dataset. Respondents have provided written approval for their involvement in the study.

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Effectiveness of Playing Origami Intervention on Improvement of Fine Motor Development Pre School Children

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Abstract

Background: One aspect of development in children is fine motor development. Delays in fine motor skills can make children have difficulty exploring the environment, barriers to learning, lazy writing, lack of creativity. This study aims to determine the effectiveness of playing Origami interventions to improve preschool children’s fine motor skills in PAUD Aisiyah VI Bengkulu City.

Study Design: This research is a Quasi-Experimental study with a research design of “one group pretest and post-test without control group design”, a sample of 26 preschool children using purposive sampling. The intervention was given for two weeks as many as six meetings with 30 minutes per session. Data on children’s fine motor development were collected using the DDST II screening format.

Results: Analysis using the Wilcoxon test obtained ρ value 0.000 (α 0.05). In conclusion, Origami play intervention is statistically effective in improving the fine motor skills of preschool children.

Conclusion: Teachers and parents should pay more attention to and increase the stimulation of preschool children’s fine motor skills by facilitating children to play folding, sticking, and paper cutting more often.

Keywords: Origami, Playing, Fine Motoric, Preschool Children

Introduction

Children are unique individuals, always cheerful, full of abilities, optimistic, and curious in their development. Children are the hope for the nation’s future and as the next generation in the future1. The child development aspect is an essential part of achieving the Sustainable Development Goals (SDGs) target. Child development means talking about the growth and development of children2.

One crucial aspect of development is fine motor development, which involves the smaller muscles and coordination of the eyes, hands, and fingers. This skill requires great precision. The development of children’s motor skills depends on the child’s maturity level. At the age of preschool, children’s fine motor skills begin to develop where children can draw and write, color, paint, eat with a spoon, color with fingers, and cut3.

A child can experience developmental delays in only one developmental domain or more. About 5 to 10% of children are thought to have developmental delays. It is estimated that about 1-3% of children under five years of age have general developmental delays (GDD)4. Motor development needs to be a severe concern for parents and teachers because this development is sometimes rarely noticed.

Several studies have shown that preschool children’s fine motor skills are related to cognitive abilities, mathematical abilities, and even literacy abilities of children in the future. Children who have not acceptable motor delays will have difficulty exploring the environment and experience obstacles in learning, writing, and creativity. As a result, children feel inferior,
doubters, and often misgivings about their environment. Furthermore, children can experience concentration and socializing problems when the child is in elementary school later. Fine motor development requires adequate stimulation. Some play activities that can be done in preschool children can be applied to practice fine motor skills such as playing puzzles, cutting, pasting pictures, sewing, paper folding or origami, coloring, finger painting and playing toy wax or plasticine. Origami play activity is a technique and art for folding a sheet of paper into an object or shape. The folding article appears to be a simple action but requires planning, precision, coordination of the eyes, and fingers, including muscle strength, sense of taste, and touch. Origami is the art of paper folding originating from Japan that trains children’s creativity, fine motor skills, coordination of the child’s brain and hand muscles, trains the accuracy and strength of the fingers, and teaches children’s patience.

The initial survey conducted by researchers at PAUD Aisyiyah VI Bengkulu City through interviews with teachers and three parents, from the three parents it was found that if their children had never been tested for development, the results of interviews with teachers were obtained that in PAUD children are rarely invited to play origami, children usually geared towards drawing and coloring with crayons.

Researchers are interested in knowing the effect of Origami play interventions on children’s fine motor development. Given that the right fine motoric product will affect children’s outcomes in the future, it should be stimulated frequently. The purpose of this study was to determine the effectiveness of playing Origami interventions to improve the fine motor skills of preschool children in PAUD Aisyiyah VI Bengkulu City.

**Materials and Methods**

This research is a quantitative research using the Quasi Experiment method with the research design “one group pretest and post-test design,” the study was conducted at PAUD Aisyiyah VI Bengkulu City during implementation March-August 2020. The sample in this study were 26 children who were taken using the purposive technique. Sampling. Data on the good motoric development categories of children were obtained using the DDST II (Denver Development Screening Test II) format.

Before the intervention was carried out, the researcher first measured the child’s fine motor development according to its age. After being given the intervention, the researcher again estimated the child’s fine motor development using the same format. The materials needed for the intervention are origami paper of various sizes and colors, a ruler, and scissors. Playing time is 30 minutes, carried out in 6 meetings for two weeks, origami is made to adjust to objects or animals that are liked by children.

**Result**

**Category of Children’s Motor Development Level Before and After Origami Play Intervention**

Table 1. Fine motor development level category of preschool children before intervention playing origami

<table>
<thead>
<tr>
<th>Fine motor development level category</th>
<th>Frequency distribution before the intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untestable</td>
<td>3 11,5%</td>
</tr>
<tr>
<td>Suspect</td>
<td>13 50,0%</td>
</tr>
<tr>
<td>Normal</td>
<td>10 38,5%</td>
</tr>
<tr>
<td>Total</td>
<td>26 100 %</td>
</tr>
</tbody>
</table>

From table 1 above, it is known that before the intervention of playing origami, most of the children, namely 13 children (50.0%), were included in the suspect category and ten children (38.5%) who are included in the variety of acceptable motor development standard.

**Table 2. Fine motor development level category of preschool children after intervention playing origami**

<table>
<thead>
<tr>
<th>Fine motor development level category</th>
<th>Frequency distribution after an intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untestable</td>
<td>5 19,2%</td>
</tr>
<tr>
<td>Suspect</td>
<td>21 80,8%</td>
</tr>
<tr>
<td>Total</td>
<td>26 100 %</td>
</tr>
</tbody>
</table>
In table 2 above, it is known that after the intervention of playing origami, most preschool children have the category of suspect fine motor development, and there are 21 children (80.8%) who are included in the category of normal fine motor development.

**Data Normality Test**

<table>
<thead>
<tr>
<th>Research Variables</th>
<th>N</th>
<th>Shapiro Wilk Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptable motor development categories before Playing Origami Interventions</td>
<td>26</td>
<td>0,000</td>
</tr>
<tr>
<td>Acceptable motor development level categories before Playing Origami Interventions</td>
<td>26</td>
<td>0,000</td>
</tr>
</tbody>
</table>

Based on table 3 above, it is known that all data are not normally distributed (ρ value <0.05) so that the bivariate test taken is a non-parametric test, the test is the Wilcoxon test to see the effect before and after giving intervention.

**The Effect of Playing Origami Interventions on the Improvement of Fine Motor Skills of Children Pre-School**

<table>
<thead>
<tr>
<th>Skor Post Intervensi Finger Full - Skor Pre Intervensi Finger Full</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Ranks</td>
<td>0a</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>14b</td>
<td>7.50</td>
<td>105.00</td>
</tr>
<tr>
<td>Ties</td>
<td>12c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From Table 4 above, it is known that no children experienced a decline in the category of fine motor development skills before and after the intervention. Fourteen children had improved good motor development categories, and 12 children had unchanged acceptable motor development categories.

**Table 5. The Effect of Origami Playing Interventions on the Improvement of Fine Motor Skills**

<table>
<thead>
<tr>
<th>Variabel</th>
<th>N</th>
<th>Mean</th>
<th>Standar Deviasi</th>
<th>ρ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine motor development level category before playing Origami intervention</td>
<td>26</td>
<td>2.27</td>
<td>0.667</td>
<td>0.000</td>
</tr>
<tr>
<td>Fine motor development level category after playing Origami intervention</td>
<td>26</td>
<td>2.81</td>
<td>0.402</td>
<td></td>
</tr>
</tbody>
</table>

From the table above, it is known that the average score category for the level of fine motoric development before the Origami Play Intervention was 2.27 and increased to 2.81 after the intervention, with a ρ value of 0.000 (α = 0.05).
It can be concluded that playing Origami interventions are effective in improving fine motor development of preschool children.

**Discussion**

**Category of Children’s Motor Development Level Before and After Playing Origami Intervention**

From table 1 above, it is known that there are three children (11.5%) who have the untestable fine motor development category, 13 children (50.0%) are included in the suspect category. There are ten children (38.5%) included in the fair motor development category origami play.

There are several factors according to researchers that cause children to get suspect and untestable results at the time of the first test (pretest) in this study; the first is the effect of the child’s age when tested and the child’s experience of the components tested based on the child’s age, given the Denver II test component—based on the child’s age line. This is by the research results Yılmaz (2016), where the difference in the child’s age when tested affects the social skills, language, and movement of the child. The child’s stimulation and environmental factors also play a role in determining this test. Parents’ role is an essential part of providing early stimulation for children’s intelligence and development in the future.

Children who get more developmental stimulation from parents and caregivers will make their children develop better, considering that development in early childhood is something that can be trained, stimulated and intervened as often as possible. Parents and or caregivers’ involvement in providing stimulation to children is essential. Given the development of children’s motor skills, both gross and fine motor skills that develop well from an early age, affect the development of children’s language skills during infancy and toddlerhood.

Table 2 above provides information that there were five children (19.2%) who had the suspect fine motor development category after the Origami play intervention, and 21 children (80.8%) were included in the normal fine motor development category. From this data, it is known that there has been an increase in the development of children’s good motor categories before and after giving Origami play interventions.

The product is an increase in the development of fine motor skills after giving origami play interventions, likewise with research Claudia (2018), which shows an increase in fine motor skills in PAUD children after playing origami. Origami is an activity that trains creativity, fingers, arm, and wrist movements. Origami teaches art to children, trains eye coordination and small muscles of children’s fingers so that if this stimulus is done regularly and continuously, it will introduce the child’s fine motor skills.

Based on table 4, the mean rank of the increase in fine motor skills above is known as no children who experience a decrease in the category of adequate motor development ability before and after the intervention (Negative Ranks). Fourteen children had an increase in the fine motor development category (Positive Ranks), and 12 children had the fine motor development category that did not change (Ties). The 14 children who experienced an increase in most of the suspect development level categories increased to normal. In comparison, the 12 children who were recorded as not sharing the development category mostly came from the normal development category when measured before the intervention, so that there were no changes in the level of development. The Wilcoxon test results obtained a $p$ value of 0.000 ($\alpha$ 0.05), which means that playing origami effectively improves children’s fine motoric development. Increase in the mean score of the level of child development category.

During the second test (post-test) using the Denver II format, it was seen that the child succeeded (Passed) in answering and carrying out the examiner’s orders, this increased ability was because the child had been trained in fine motor skills using paper folding media or origami. The coordination of the eyes, wrists, and fingers is essential when folding paper, origami makes the development of the fingers and arms increase. Playing origami also requires children to be patient, repeat if they are wrong, and try again, so that in addition to increasing psychomotor abilities, children’s thinking and cognitive skills also develop. This is what makes children’s motor skills increase compared to before.

Given that fine motor development is an essential part of child development. The story of the preschooler’s small muscles and to carry out functions such as writing,
using a spoon, coloring, drawing. Good fine motor skills affect the level of children’s development in the future.\textsuperscript{13}

When the right stimulus is given continuously from an early age, the child will get used to practicing fine motor skills. At an early age, children’s brain development increases rapidly. The provision of origami playing interventions and their effectiveness in improving children’s fine motor skills is in line with the results of research conducted by Widayati (2019) there is an increase in the child’s fine motor skills after being given paper folding play intervention.\textsuperscript{14}

**Conclusion**

Based on the results of the research and discussion above, it can be concluded that the intervention playing origami is effective in improving the fine motoric development of preschool children in PAUD Aisyiyah VI, Bengkulu City. The Wilcoxon test results obtained a value of \( \rho \) value 0.000 (\( \alpha \) 0.05). Teachers and parents should pay more attention to and increase the stimulation of fine motor skills of preschool children by facilitating children to play folding, sticking, and paper cutting more often.

**Conflict of Interest:** The authors declare that there is no conflict of interest.

**Source of Funds:** This research is a Beginner Lecturer Research with funding sources from Ristekdikti grants.

**Ethical Clearence:** Health Research Ethics Committee, Health Polytechnic of Health Ministry Bengkulu

**References**


The *ITGA2B* gene Polymorphism Associated with Glanzmannthrombasthenia in Sample of Iraqi Patients

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**Abstract**

Case control study was used, with healthy individual control (n=20) and Glanzmannthrombasthenia patients (n=15). The diagnosis was depending on the clinical, hematological parameters and molecular analysis. All patients were severe bleeding symptoms with normal Hemoglobin, and platelets count and prolonged bleeding time.

It was successfully identified three SNP in *ITGA2B* gene the first SNP c.2653 T>G (rs5911) was presented with three genotypes (TT, TG and GG). The genotype frequencies of TT in control group (60.0 vs. 90.0%) show significant difference (p ≤0.05) compared with GT patients. It was also noticed the frequency of mutant allele (G) revealed a significant difference (p ≤ 0.01) in GT patients compared with controls group (33.3 vs. 7.5%; OR = 6.17; EF = 0.44; 95% C.I. = 1.55 - 24.53) respectively.

The c.641T>C (rs137852911) was given with three genotypes (TT, TC and CC). The frequencies of these genotypes show non-significant difference (p ≤0.05 between control and GT patient, while the mutant allele (C) show significant difference (p ≤ 0.01) in GT patients compared with controls group (10 vs. 7.5%; OR = 1.37; EF = 0.11; 95% C.I. = 0.26 – 7.14).

The c.6438G>A showed three genotypes (GG, GA and AA) with two alleles (G and A).

The frequencies of these genotypes GG (60 vs. 95 %), GA (33.3 vs. 5.0%) and the third genotype (AA) 26.6 vs. 0% show significant difference (p ≤0.05) between control and GT patient. It was also found the mutant allele (A) revealed a significant difference (p ≤ 0.01) in GT patients compared with controls group (33.3 vs. 2.5%; OR = 11.87; EF = 0.35; 95% C.I. = 1.41 – 99.80) respectively.

**Keywords:** Glanzmannthrombasthenia, genotypes, revealed, bleeding time, frequencies

**Introduction**

Glanzmannthrombasthenia (GT) is a rare, inherited disorder of platelet function characterized by absence of platelet aggregation, and prolonged mucocutaneous bleeding tendency, caused by qualitative or quantitative defects of the platelet membrane integrin αIIbβ3(¹, ²).

The molecular basis of GT is linked to qualitative and/or quantitative abnormalities of αIIbβ3 integrin that between binding of the adhesive proteins lead to aggregating platelets and clot formation at position of wound (¹). Patients have been separate into three groups: type I where platelets absence αIIbβ3 (have <5% of the normal platelet content), type II with residual αIIbβ3 (5–15%) that may be functional or not, while in type III with αIIbβ3 fails to function despite platelets possess upto 100% of normal levels (³).

Many Heterozygous or Homozygous mutations found in *ITGA2B* gene and these mutations are led to splice defects and stop codons truncate or lead to the loss...
mRNA synthesis(3).

It was reported that GT appear in many ethnic populations such as Iranians, Indians, Iraqi Jews, Jordanian Arabs, and Palestinians in high frequency(4).

As a mention above this study was aimed to investigate the ITGA2B gene polymorphism in a sample of Glanzmann thrombasthenia Iraqi patients.

Materials and Methods

Case control study was used, with healthy individual control(n=20) and Glanzmann thrombasthenia patients(n=15) were recruited at the Division of pediatric teaching hospital in Baghdad and Karama Teaching Hospital in wassit. Both laboratory data and clinical of every patient were gathered from their clinical records which included gender, age, age at determination, period of beginning, bleeding manifestations, and consanguinity of parents. Samples of patients and the control group were collected in EDTA, tubes, and hematology tests (Hb and platelets count) were performed by Coulter automated analyzer and the bleeding time examination was done.

Isolation of DNA from the EDTA blood of patient samples and control groups using DNA Kit from Promega. regions of ITGA2B gene were amplified by PCR using primers. Which was designed using the program PRIMER3. Genomic DNA was used as the template for 25 PCR reaction system containing 12 µM of Master Mix, 10 µM each of oligonucleotide primers, 8.5µM Nuclease Free Water and 2 µM Taq DNA Polymerase. Thermocycling conditions of primers F5'-TATGTCAGTGCTTGGAGCCC-3' and R 5'-CAGAGAGCCTGCTCACTACGA-3',andprimers F5'-GTGCTTTGGGTACAAGAATG-3' and R 5'-TCCTCCACCAAGTCTCTAATAATC-3' were as follows: an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation , annealing, and extension at 95 °C for 45 s, 60 °C for 45 s, and 72 °C for 45 s Respectively. A final extension step (72 °C, 7 min) was performed at the end. other primers F 5'-CTCCAGGTGATGAGACCGG-3' and5-R5'-TCTGGAATGCCGGTGTACC-3' have same thermocycling conditions but have different annealing temperature (65 °C).

Using ABI3730XL, a Sanger sequencing analysis was performed on the PCR product by Macrogen Corporation - Korea, and the results were analyzed using a genious program.

All data was analyzed statistically by using SPSS software (Statistical Package for Social Sciences) version 20 and p-values were set at 0.001. Data are presented as Mean ± SD (standard deviation). The results were analyzed using analysis of variance (ANOVA).

Depending on Hardy-Weinberg equilibrium, the difference between the expected and observed frequencies of the genotype was determined as well with respect to the expected and observed alleles at the control and patient groups. Alleles and genotypes of ITGA2B genes were presented as percentage frequencies, and significant differences between their distributions in GT patients and controls were assessed by two-tailed Fisher’s exact probability (P). In addition, odd ratio (OR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between ITGA2B and ITGB3 alleles and genotypes with the disease, these estimations were calculated by using the WINPEPI computer programs for epidemiologists, which is available free online at http://www.brixtonhealth.com.

Result

GT was diagnosed depend on of Clinical signs and data of each patient. The results of the hemoglobin test and platelet count were within the normal range. Where were the results of an examination HB mean 11.7±1.7 of patients and mean 12±1 of control group. While the average platelet count test values were mean 217.1±9.2 of patients and mean 296±9.9 of control group. The platelet count and the hemoglobin parameters and indexes measured in the current work were not significantly different ( p<0.001) between Glanzmann patients, and control groups.

Whereas, there was a significant difference between the values of bleeding time result (mean 13.9±3.2) for patients compared to the control group (mean 4.7±1.5).

The results showed that after ethidium bromide staining, a main band (length 980 bp) appeared on the gel, which represented the amplification of the restricted
region from 44375568 to 44376560 in the ITGA2B gene containing exons 23, 24 and 25 with introns between these exons. The other result of the amplification of the DNA fragments by the primers is the region between 44384919 and 44385928 with a band of 1000 bp on the gel and the exons 3, 4, 5, 6 and 7. The DNA fragment between 44383196 and 44384189 was amplified by PCR and primer set. The length of the product DNA fragment was 990 bp, including exons 11 and 12 figure(1).

Sequencing analysis can detect a total of 3 different mutations in the ITGA2B gene. c.2653 T>G mutation is the replacement of nucleotide T allele in position chr17:44375697 (GRCh38.p12) in exon 24 with G allele, which causes the amino acid isoleucine to become a serine substitution at position 874 of the GPIIb figure (2). c.641T>C mutation at position chr17: 44385193 (GRCh38.p12) revealed that the mutation changed T>C in exon 6 and caused the amino acid leucine to become proline at position 214 in the polypeptide chain figure (3). c.6438G>A mutation of chr17:44383668 (GRCh38. p12) of exon 12 was detected by sequencing analysis, and caused the amino acid at GPIIb 345 to replace methionine with isoleucine figure (4).

Figure 1: PCR products of amplification fragment DNA on 2% agarose gel at 70 voltages for one hour of ITGA2B gene in the confined areas: A- from 44375568 to 44376560 which contains exons 23, 24, and 25 of GT patients with band have 980 bp. B- from 44383196 to 44384189 which contains exons 11, and 12 of GT patients with band have 990 bp. C- from 44384019 to 44385928 which contains exons 3, 4, 5, 6 and 7 of GT patients with band have 1000 bp. Marker: ladder marker DNA 100 bp.
Figure 2 Nucleotide sequencing analysis of ITGA2B gene. Results for nucleotides are shown for the GT patient. The nucleotide substitution T>G at position 2653 in exon 24 and the corresponding amino acid change from Ile874 (ATC) to Ser874 (AGC) are indicated.

c.2653 T>G (rs5911)

Figure 3. Nucleotide sequencing analysis of ITGA2B gene. Results for nucleotides are shown for the GT patient. The nucleotide substitution T>C at position 673 in exon 6 and the corresponding amino acid change from Leu 214 (CTT) to Pro 214 (CCT) are indicated.

c.641T>C (rs13782911)
The SNP (c.2653 T>G (rs5911) at position 2653 in exon 24 was presented with two alleles (T and G) and three genotypes (TT, TG and GG). These genotypes showed deviation from HWE in GT patients, because there was a significant difference between the observed and expected genotype frequencies (p ≤ 0.01), it was also noticed a deviation from HWE in controls treatment with significant difference (p ≤ 0.01).

Comparing GT patients to controls revealed that genotype frequencies of TT (60.0 vs. 90.0%) and TG (13.4 vs. 5.0%), while the third genotype (GG) was observed with a frequency 26.6 vs. 5.0% in patients and control group respectively. The genotype TT in control group was significant difference (p ≤ 0.05) compared to GT patient, but TG and GG were show non-significant difference (p ≤ 0.05) compared between control and GT patient. It was also noticed the frequency of mutant allele (G) show a significant difference (p ≤ 0.01) in GT patients compared to controls (33.3 vs. 7.5%; OR = 6.17, EF = 0.44; 95% C.I. = 1.55 - 24.53) that mean (G) allele (OR = 6.17) is risk factor associated with GT patients and negatively associated with healthy subject, while in the control group the frequency of (T) alleles show a significant difference (p ≤ 0.01) compared to GT patients (92.5 vs. 66.6; Reciprocal of OR = 6.17, EF = 0.932; 95% C.I. = 0.04 - 0.65). In this case the (T) allele associated with healthy subject and considered as protective factor, but in GT patients group was negatively associated with Reciprocal of OR = 6.17 (Table).

The SNP rs137852911 (c.641T>C) at position 9313 in exon 6 was presented with two alleles (T and C) and three genotypes (TT, TC and CC). These genotypes showed deviation from HWE in GT patients, because there was a significant difference between the observed and expected genotype frequencies (p ≤ 0.01), it was also noticed the genotypes frequencies (TT, TC and CC) show deviation from HWE in controls group with significant difference (p ≤ 0.01).

Comparing GT patients to controls revealed that genotype frequencies of TT (86.6 vs. 90.0%) and TC (6.6 vs. 5.0%), while the third genotype (CC) was observed with a frequency 6.6 vs. 5.0%. These genotypes were non-significant difference (p ≤ 0.05) compared between control and GT patient. It was also noticed the frequency of mutant allele (C) show a significant difference (p ≤ 0.01) in GT patients compared with controls (10 vs. 7.5%; OR = 1.37; EF = 0.11;
95% C.I. = 0.26–7.14), while in the control group the frequency of (T) allele show a significant difference (p ≤ 0.01) compared to GT patients (90 vs. 92.5; Reciprocal of OR = 1.73, EF0.71; 95% C.I. = 0.14–3.8). In this case the (T) allele associated with healthy subject and non-associated with GT patients (Reciprocal of OR = 1.73) and considered as protective factor (Table).

The c.6438G>A at position 6582 in exon 12 was presented with three genotypes (GG, GA and AA) and two alleles (G and A). These genotypes showed deviation from HWE in GT patients, because there was a significant difference between the observed and expected genotype frequencies (p ≤ 0.01), it was also noticed a deviation from HWE in controls treatment with significant difference (p ≤ 0.01).

Comparing GT patients to controls revealed that genotype frequencies of GG (60 vs. 95%), GA (33.3 vs. 5%), and The third genotype (AA) 26.6 vs. 0% and The third genotype (AA) 26.6 vs. 0%. The GA and AA genotypes were show non-significant difference between healthy subject and GT patient, while the genotype GA was significant difference (p ≤ 0.05) compared between control and GT patient. It was also noticed the frequency of mutant allele (A) show a significant difference (p ≤ 0.01) in GT patients compared to controls (33.3 vs. 2.5%; OR = 11.87; EF = 0.35; 95% C.I. = 1.41–99.80) that mean (A) allele is risk factor associated with GT patients and negatively associated of healthy, while in the control group the frequency of (G) allele show a significant difference (p ≤ 0.01) compared to GT patients (66.6 vs. 97.5; Reciprocal of OR = 11.87; EF0.98; 95% C.I. = 0.01-0.71) and positively associated of health and negatively associated of GT patients (Table).

### Table: Genotype and allele frequencies and epidemiological parameters for Iraqi GT patients and controls group.

<table>
<thead>
<tr>
<th>ITG42B gene</th>
<th>GT patient (15)</th>
<th>Control (20)</th>
<th>Epidemiological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>Observed (%)</td>
<td>Expected (%)</td>
<td>HWE P value</td>
</tr>
<tr>
<td>rs9511</td>
<td>TT 9 (60)</td>
<td>18 (90)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>TG 2 (13.4)</td>
<td>1 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>GG 4 (26.6)</td>
<td>1 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>T 20 (66.6)</td>
<td>37 (92.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>rs137852911</td>
<td>TT 13 (86.6)</td>
<td>18 (90)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>CT 1 (6.6)</td>
<td>1 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>CC 1 (6.6)</td>
<td>1 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>T 27 (90)</td>
<td>37 (92.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>c.6438G&gt;A</td>
<td>T 3 (10)</td>
<td>3 (7.5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>GG 9 (60)</td>
<td>19 (95)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>GA 5 (33.3)</td>
<td>1 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>AA 1 (26.6)</td>
<td>0 (0)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>G 23 (66.6)</td>
<td>39 (97.5)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>A 7 (33.3)</td>
<td>1 (2.5)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

EF: Etiological fraction, PF: Preventive Fraction, OR: Odds ratio, C.I.: Confidence interval

HWE: Hardy-Weinberg Equilibrium, *: Reciprocal of odds ratio
Desiccation

GT has been reported among Iraqi patients by several groups (5, 6), including a series of 12 patients from Iraqi-Jewish which was described in 1991(7), and other group from Iraqi-Jewish was described in 2001 (8). The average age of patients was 20 years in this study and this is consistent with Al-Rahal and Giath 2015, which stated that this disease affects children and young adults. Most of the cases were caused by the consanguineous marriage to the parents. Consanguineous marriage is a common behavior in Iraqi populations, also prevalent in some populations, such as Iranians, Saudi Arabia, and Southern Indians (6). Consanguineous marriage between parents is an important risk factor for GT (5).

The normal level of Hb was expected in Glanzmann, because the defects of these diseases directly affect platelets, not directly affect the level, number and nature of red blood cells. However, in some samples, the level of hemoglobin has decreased, especially in some women, the common problem that troubles women with Granzman is menorrhagia (9). The platelet count results of all patients and the control group were normal, and the difference was not statistically significant and this is result correspond with Sabhan et al. 2017.

Regarding prolonging the bleeding time of patients (n = 15), statistical analysis showed a significant difference (p ≤ 0.01) compared with the control group. Prolonged bleeding time suggests delayed primary hemostatic thrombosis due to poor platelet aggregation (5).

As a mention above my be Rs5911 T > G considered as missense mutation that causes the substitution of isoleucine at position 843 of the GPIIb heavy chain with a serine amino acid. Therefore, rs5911 may work by affecting post-fibrinogen binding events, including cytoskeletal reorganization and clot retraction, and in some way affect the stability of platelet/fibrinogen interaction (10) depending on this finding the (G) allele considered as risk factor associated with GT patients while the (T) allele associated with healthy subject and considered as protective factor.

The c.641T>C mutation results in the creation of a proline-glycine-alanine-proline (PGAP) sequence at the amino-terminal end of the b-turn structure. The rigidity of the two proline residues linked by flexible glycine and alanine residues may create a twist in the secondary structure affecting the conformation of the b-turn. The inability of mutant GPIIb/IIIa receptors to be recognized by conformation-dependent antibodies, to be activated into a high-affinity ligand binding conformation, and to adhere to immobilized fibrinogen suggests that this structural alteration affects ligand-binding and that the Leu214Pro mutation has either an indirect or direct effect on this site (11). In this study, it was show the (C) allele considered as risk factor associated with GT patients while the (T) allele associated with healthy subject and considered as protective factor.

c.6438G>A mutation in exon 12 was lead to substitution amino acid Methionine to Isoleucine acid at position 345 of the GPIIb. The change in the amino acid Methionine to Isoleucine may lead to a change in the structure of the receptor and thus affect the function of the receptor.

In this result, it was found the (A) allele considered as risk factor associated with GT patients while the (G) allele associated with healthy subject and considered as protective factor.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References


Isotherms, Kinetics and Thermodynamic Studies for Removal the Valium from Stomach and Intestine Fluids via Adsorption on Egg Shells Powder

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Abstract

Drug abuse or overdose in accidents is a serious social issue. This study concerned the adsorption behaviour of the Valium drug in both simulated gastric and intestinal pH onto the untreated eggshell powder by using UV spectroscopy. The effects of various experimental parameters such as contact time, pH-solution, an adsorbent dosage of Valium and temperature range (300-320k) on the adsorption capacities have been investigated. The suitability of Langmuir and Freundlich adsorption models to the experimental equilibrium data at different solution temperatures were calculated. The adsorption was well described by the Freundlich isotherm model and Langmuir, $S_1$ for the stomach and $S_2$ for the intestine according to the Giles classification. Thermodynamic parameters such as $\Delta H^\circ$, $\Delta G^\circ$, and $\Delta S^\circ$ results showed that the adsorption of the drug increases by increasing the temperature in the stomach solution, i.e., the endothermic reaction decreases by increasing the temperature at the intestinal solution, i.e., the reaction is exothermic. Experimental data were also tested in terms of adsorption kinetics, the results illustrated that the adsorption process was following pseudo-second-order kinetics in the stomach solution and first order in the intestinal solution.

Key words: Adsorption, Langmuir, Freundlich, Kinetics, Thermodynamics

Introduction

Sorption and adsorption process have been extensively studied as a low cost, effective method for removing a wide variety of hazardous materials from aqueous solutions. Principle considerations in the manufacturing of a drug product include the therapeutic goal, the position of application, and systemic drug sorption from that position. The drug should preferably be entirely and steadily absorbed from the Stomach and intestine sites. Valium is a trading name for Diazepam drug which widely used to treat various types of epilepsy, insomnia, and anxiety, and muscular spasms. Overdose Valium can have serious consequences that can cause a number of side effects and hence death in some cases.

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Figure(1): Structure of Valium
Diazepam is one of the most widely prescribed 1,4-benzodiazepine. The consumption of Valium along with other substances such as alcohol is known to increase its sedative effects and its increased absorption rate. To alleviate these effects a range of analytical approaches has been investigated to determine the adsorption of diazepam on the surface of safe substances like an eggshell powder. Adsorption is a highly effective, economical, promising method that applied for purification wastewaters. Different conventional and non-conventional adsorbents have been used such as Hen egg-shell. Hen egg-shell typically consists of ceramic...
materials constituted by a three-layered structure, namely the cuticle on the outer surface. The chemical composition of the eggshell has been reported by weight as follows: calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%), and organic matter (4%) (4). Sorption–desorption studies were carried out for five cycles by Vijayaraghavan and et al. using domestic eggshell to remove copper from aqueous solution in an up-flow packed column (5). Another study has been described the adsorption isotherms by Otun et al. using Powdered eggshell (particle size 63 μm) as an adsorbent to remove three metal ions Pb (II), Ni (II), and Cd (II) from aqueous solution and from natural water in a batch process (6). Based on reported research in the literature, it was found that there was a high sorption capacity as compared to other adsorbents for a low-cost sorbent eggshell to remove Cr (III) ions from aqueous solutions (7). In this work, the equilibrium adsorption characteristics of Valium on the crushed eggshell’s surface have been evaluated in batch experiments. The adsorption isotherms, temperature effect, pH adjustment, Kinetic measurements (determined by the pseudo-first-order, pseudo-second-order) and thermodynamic parameters have been calculated.

Waste eggshells have used as adsorbate due to their recycling; it has the potential to reduce environmental pollution while acting as a low cost, safe effective material to reduce Valium absorption by the Digestive.

Experimental methods of adsorption

Chemical materials

Hydrochloric acid (Sigma Aldrich, 99.0%), Sodium chloride (Sigma Aldrich, 99.0%), Sodium hydroxide (Sigma Aldrich, 97.0%), Potassium phosphate (Alfa Aesar, 97%).

Adsorption characterization

All spectral and absorbance measurements were conducted on Shimadzu Uv-Vis 1700 digital double beam recording spectrophotometer (Italy) using 1cm glass cells. this technique provides information about the adsorption of adsorbate on the adsorbent surface whether it is physical or chemical adsorption. The results of the current study show the susceptibility of the eggshells surface to the adsorption of the Valium by using UV spectroscopy. A digital pH meter 720 WTW 82362 has also been used to adjust the acidity or alkalinity of a solution.

Absorbate solutions preparation

Stock solutions of Valium were prepared by dissolving 50 g in distilled water to give a concentration of 100 mg/L and that diluted with distilled water when necessary to prepare other concentrations. The drug may be precipitated with pH between (7.2 – 7.4). To assess the suitability of the approach, the selected pH is important to consider. In the stomach, the pH is about 1 - 2 and in the duodenum, the pH is between 5 to 7.5. so, the degree of solubility is also likely to be influenced as the drug passes through the intestines (8).

To prepare stomach fluid, 2g of sodium chloride and 7ml of hydrochloric acid have been dissolved in distilled water until the pH solution adjusted to 1.2. To prepare intestinal fluid, 0.89 g of sodium hydroxide and 6.8g of anhydrous potassium phosphate (KH₂PO₄) have been dissolved in distilled water until the pH solution adjusted to 6.8.

Preparation of biological sorbent

Eggshells were collected from kitchen waste and washed thoroughly in running water for several times and then rinsed with deionized water until no foreign material remained behind. After washing with distilled water, the sample was dried in an oven at 60 °C for two hours to drive off any moisture. The dried eggshells were crushed and milled in an agate mortar and pestle to form a fine powder with a high specific surface area, then passed through a (200 μm) sieve, the portion which passed through the mesh is retaining in a tight container. The chemical compounds of the eggshell are: \{%W:Na₂O=0.489, MgO=0.845, Al₂O₃=0.055, SiO=0.010, P₂O₅=0.181, SO₃=0.747, K₂O=0.50, CaCO₃=97.015, Fe₂O₃=0.029, SrO=0.140, Cl=0.138\}.

Sorption Experiments

Series of batch adsorption tests were conducted to determine the effects of contact time, initial concentration of the drug dosage, amount of adsorbent, and operating temperature on adsorption performance. Therefore, various amounts of egg-shells (0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, and 1g) were introduced into a series of conical
flasks each filled with 25 mL of Valium solution with 40 ppm at pH 1.2, and pH 6.8 to determine the amount of adsorbent. The flasks were then placed in a thermostat water bath shaker and agitated up to a total contact time of 30 min at a fixed agitation speed of 90 rpm. Drug concentration in the samples was analysed by atomic absorption spectrophotometer. Initial drug concentration was determined by shaking (25mL) of different initial concentrations (10–50 ppm) of Valium solution placed in five conical flasks with 0.1 g of eggshells at (pH = 1.2), and pH 6.8, for 30 min at 310 k. Equilibrium data were obtained after the solution was filtered and analyzed. In addition to these experiments, the effect of contact time on the drug adsorption was also investigated. Batch adsorption tests were carried out at different contact time from 10 to 120 minutes, 30 min was found to be the best period of contact at equilibrium.

Another experiment, 25 mL of drug solution with a concentration of 40 ppm was added to a fixed amount of adsorbent (0.1 g of eggshells in (50 mL) conical flask and agitated in thermostat shaker with a speed 90 cycle/minute until reaches equilibrium after (30 min) at 310 k temperature, then the solution was filtered and analysed by measuring absorbance at a maximum wavelength of (358 nm) and (413 nm) for stomach and intestinal solutions respectively. The residual Valium concentration in the supernatant was determined using a UV/Vis instrument. The amount of Valium adsorbed was calculated by measuring the difference in concentration between samples that were obtained at two consecutive time intervals over the period of the adsorption experiment.

Again, the experiment was repeated at different temperatures (300 and 320) k to investigate the operating temperature on adsorption performance and to calculate thermodynamic parameters (ΔH°, ΔG°, ΔS°). The sorption capacity was calculated by the following equation (10,11)

$$Q_e = \frac{(C_0-C_e) V_{sol}}{M}$$

Where: $C_0$: is the initial concentration (mg/L), $C_e$: is the equilibrium concentration (mg/L), $V_{sol}$: is the volume of drug solution (L), and $M$: is the weight of eggshells (g).

The experimental data were calculated to describe adsorption equilibrium isotherms by using two main theories, Langmuir and Freundlich models. The main assumption of the Langmuir adsorption model is that the surface of the adsorbent is covered with a monolayer of adsorbed molecules. Langmuir isotherms which represented by the linear equation:

$$\frac{C_e}{q_e} = \frac{1}{a K_l} + \frac{1}{a C_e}$$

Where $a$ and $K_l$ represent Langmuir’s constants related to limiting adsorption capacity when the surface is fully covered with a monolayer adsorbate and rate of adsorption respectively. $C_e$ (mg/L) is the equilibrium concentration of the adsorbate, $q_e$ (mg/g) is the amount of Valium adsorbed per unit mass of adsorbent. Langmuir’s equation assumes the formation of one layer of adsorbate molecules on the surface while the Freundlich isotherm explains the behaviour of adsorption by the formation of more than one layer and consider the heterogeneity of the surface. The linear form of Freundlich isotherm is:

$$\log Q_e = \log K_f + \frac{1}{n} \log C_e$$

Where $K_f$ and $n$ are Freundlich constants that depend on the nature of the adsorbent and the gas at a particular temperature which characteristics of the system, including the adsorption capacity and adsorption intensity, respectively (12). In other words, $K_f$ can be defined as the distribution coefficient and represents the quantity of drug adsorbed onto eggshells at equilibrium concentration.

The equilibrium constant ($K_{eq}$) for the adsorption process at each temperature is calculated by using the relationship as follow:

$$K_{eq} = Q_e \frac{W_{ts}}{V_{so}} \frac{(C_0 - C_e) V_{sol}}{M}$$

Where ($W_{ts}$) is the weight of eggshell, ($V_{so}$) is the volume of drug solutions.

2.5.1. Thermodynamic calculations

Thermodynamic parameters (ΔH°, ΔG°, ΔS°) were calculated using three different temperatures 300, 310, 320 K to evaluate the feasibility of the adsorption process. The change in free energy ($\Delta G^0$) could be calculated at absolute temperature (T) with the gas constant 8.314 J.mole$^{-1}$.K$^{-1}$ as shown in following equation:

$$\Delta G^0 = -RT \ln K_{eq}$$
\[ \Delta G^o = -RT \ln K \]  \hspace{1cm} (6)  

\[ \Delta G^o = 2.303 RT \log \frac{Q_e}{C_e} \]  \hspace{1cm} (7)

Vant Hoff’s\(^{(13)}\) and Gibbs equations were used to determine the heat of adsorption \(\Delta H^o\) and change in entropy \(\Delta S^o\) respectively as shown in the equations below. Where \(K\) is the equilibrium constant when \((C_e)\)-approaches zero at a certain temperature, \((X_m)\) is the maximum uptake of adsorption at a certain value of equilibrium concentration \((C_e)\).

\[ \ln K = \frac{-\Delta H^o}{RT} + \text{constant} \]  \hspace{1cm} (8)

\[ \log X_m = -\frac{\Delta H^o}{2.303 RT} + \text{Con.} \]  \hspace{1cm} (9)

\[ \Delta G^o = \Delta H^o - T \Delta S^o \]  \hspace{1cm} (10)

**Kinetics calculations**

The effect of time on Valium adsorption was investigated. Adsorption experiments were carried out at 310 k for a period of time ranged between (5-50 minutes) while other conditions were held constant. At the end of each adsorption period, the suspension was centrifuged and filtered. The residual amount of drug in the solutions after adsorption was determined spectrophotometrically, at the maximum wavelength of 314 and 358 nm for the stock solutions of intestinal and gastric fluids respectively. In order to determine the kinetics of Valium adsorption on eggshells, pseudo-first-order (PFO), pseudo-second-order (PSO) were applied. Lagergren\(^{(14)}\) suggested a pseudo-first-order equation for the sorption processes of a liquid-solid system based on the solid surface capacity. The pseudo-first-order model equation is:

\[ \log(q_e - q_t) = \log q_e - k_1 t/2.303 \]  \hspace{1cm} (11)

The other hand pseudo-second-order model is expressed as\(^{(15)}\):

\[ \frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} * t \]  \hspace{1cm} (12)

where \(k_1\) (\(\text{min}^{-1}\)), \(k_2\) (\(\text{mg} \cdot \text{g}^{-1} \cdot \text{min}^{-1}\)) is the rate constant of the model (PFO), and (PSO) respectively, \(q_t\) is the amount of solute adsorbed (\(\text{mg}/\text{g}\)) on the adsorbent at time \(t\), and \(q_e\) is the amount of adsorption (\(\text{mg}/\text{g}\)) at equilibrium. In recent years, the linear forms of the pseudo-first order PFO, and pseudo-second order PSO are the most widely used in liquid-phase sorption processes on solid surface to determine the most fitted kinetic model for the adsorption process.

**Results and Discussion**

**Valium absorption Spectra and Calibration curve**

Firstly, The UV detection wavelength at \(\lambda_{max}=314\) for the stock solutions of intestinal fluid and \(\lambda_{max}=358\) for the stock solutions of gastric fluid were defined. The calibration curve of the drug solutions has prepared to calculate the concentrations of the samples from each experiment, five different concentrations were prepared, and the absorbance was measured via UV/Vis spectrophotometer from(400 to 700nm). The maximum absorbance and calibration curves were plotted against the concentrations of Valium for the simulated gastric fluid and the simulated intestinal fluid.

**Valium Adsorption isotherms**

Equilibrium studies determine the capacity of the adsorbent and describe the adsorption isotherm by constants which values express the surface properties and affinity of the adsorbents. Equilibrium adsorption isotherms of Valium have been studied as a function of concentration. The results in figure 3 showed that the adsorption uptake increase with increasing drug initial concentration. This was because when the initial concentration increased, the mass transfer driving force would become larger, and that resulting in higher molecules adsorption. Whereas, at lower initial concentration, a high number of active vacant sites available on the eggshell’s surface were sufficient to absorb most of the Valium molecules. As observed from the results in figure(2), the shape of the isotherms was \(S_3\) in simulated gastric fluid and \(S_2\) in a simulated intestinal fluid according to Gilles classification\(^{(16)}\).

Results in figure(2) demonstrate that the adsorption of Valium continue over the initial stage of the contact time period until reaching the equilibrium. This occurrence was due to the presence of a large number of vacant surface sites was available for adsorption during the initial stage, and near the equilibrium time, the same trends of molecules adsorption were observed in the stock solutions of intestinal and gastric fluids.

Change of pH solution affects the surface charge of the adsorbents as well as the degree of ionization
of adsorbate material. The adsorption of ions such as hydrogen, hydroxyl, and other ions are affected by the pH of the solution. As the pH increases, it is usually expected that the cationic ion adsorption also increases due to increasing of the negative surface charge of adsorbents\(^{(17)}\). Benzodiazepine is a weakly basic or neutral drug\(^{(18)}\). With increasing pH values the adsorption of Valium on eggshell tends to decrease, which can be explained by the electrostatic interaction of anionic species with the negatively charged hydrolysed eggshell composite surface. Experimental data absorption was better fitted to the Freundlich isotherm more than Langmuir in a simulated intestinal fluid SIF and fitted to the Langmuir more than Freundlich in simulated gastric fluid SGF. It can be concluded that the drug was effectively adsorbed onto eggshell at both solutions with significantly higher adsorption in simulated gastric fluid.

Freundlich and Langmuir adsorption isotherm

Analysis of adsorption isotherm is essential importance to describe how adsorbate molecules interact with the adsorbent surface. Adsorption studies were performed at two pH values 1.2 and 6.8 to simulate the environments in the gastrointestinal tract (stomach and intestine). Monolayer adsorption uptake onto a surface containing a limited number of adsorption sites has been assumed by Langmuir. When \(\frac{C_e}{q_e}\) is plotted against \(C_e\), a straight line with a slope of \(1/a\) and intercept of \(\frac{1}{K_f}\) is obtained as shown in figure(2) Langmuir plots exhibited excellent \(R^2\) value compared to Freundlich plots at pH =1.2 of SGF, indicating excellent fitting of the model to the experimental data. The Freundlich isotherm is a mathematical expression for the adsorption equilibrium between liquid and solid heterogeneous surfaces. It is supposed that the powerful binding sites are occupied first and consequently, the binding strength decreases with the increasing degree of site occupation\(^{(19)}\). Figure(2) shows that the drug concentrations on the eggshell adsorbent will increase and be more than one layer as long as there is an increase in the drug concentration in the liquid. The value of (n) represents the favourability of the adsorption process and \(K_f\) is giving an indication about the quantity of drug adsorbed onto eggshell at equilibrium concentration. The slope of 1/n is a measure of the adsorption intensity\(^{(20)}\), Table(1) demonstrates the values of \(R^2\) for each isotherm in different pH and gives an indicator about the best adsorption isotherms. By comparing the results presented in this table, greater correlation \(R^2\) value for Langmuir isotherm has registered than those of Freundlich isotherm in the SGF solution. While in SIF solution, the greater correlation \(R^2\) value was for the Freundlich isotherm.

**Fig(2):** Effect of (contact time, Calibration curve, Isotherm, Freundlich) on adsorption of Valium onto eggshell powdered in both simulated gastric SGF and intestinal fluid SIF at 310 k.
Kinetic models for the adsorption process

Equilibrium of sorption rate is an important factor for the selection of adsorption kinetics that must be taken into account to determine the sorption kinetic constants. The experimental data of the drug adsorption process kinetics Pseudo-first-order PFO and the pseudo-second-order PSO kinetic equations have been investigated in this study. After the data were fitted to kinetic equations, the highest average regression coefficients ($R^2=0.881$) were obtained for the pseudo-second-order kinetic equation in SGF solution. The lowest $R^2$ value was for the pseudo-second-order kinetic equation in SIF solution (figure.3).

Equilibrium analysis of adsorption is required for evaluating the possibility or capacity of a sorbent. However, an ideal sorbent for drug control must have not only a large sorption capacity but also a fast sorption rate. (Table.1) shows, in acidic solution (pH=1.2) are likely to be second-order because of the experimental value of $q_e$ approximately equal to the theoretical value, higher value of correlation coefficient. In contrast, very low correlation coefficient was observed for the simulated intestinal fluid. Therefore, the adsorption processes followed well pseudo second-order kinetics for the simulated gastric fluid as shown in figure(3).

**Fig(3): Kinetic of Pseudo first and second Order**

**Table(1): Parameters of the Freundlich and Langmuir models extracted from experimental adsorption isotherms data at 310 k in SGF and SIF solutions, with Constants of pseudo-first and second order**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Freundlich</th>
<th>Langmuir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$K_f$</td>
</tr>
<tr>
<td>SGF</td>
<td>2.032</td>
<td>0.015</td>
</tr>
<tr>
<td>SIF</td>
<td>0.834</td>
<td>-1.360</td>
</tr>
</tbody>
</table>
Effect of temperature on drug adsorption and Thermodynamic data

Effect of temperature on adsorption studies were carried out at (300, 310, and 320K) for Valium. The sensitivity of the adsorption process towards temperature is presented in figure (4). It was found that $q_e$ decline with increasing temperature in simulated SIF fluids, which was probably related to desorption of Valium occurred at high temperatures. These results indicate that the exothermic nature of the process (negative $\Delta H$), that occurs may be due to the tendency of drug molecules to escape from the solid phase to bulk phase with an increase in temperature of the solution.(21). The thermodynamic parameters for the drug adsorption on eggshells powder at various at three different temperatures were calculated and listed in table (2). The values of free energy change $\Delta G^*$, enthalpy change $\Delta H^*$ and entropy change $\Delta S^*$ were calculated Based on the available equations to assess the thermodynamic feasibility of the process and to confirm the nature of the adsorption process, Based on the following literature available equations(22).

<table>
<thead>
<tr>
<th>Fluids</th>
<th>Pseudo-first-order</th>
<th>Pseudo-second-order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$q_e$ mg/g</td>
<td>$k_1$ min$^{-1}$</td>
</tr>
<tr>
<td>SGF</td>
<td>7.362</td>
<td>0.172</td>
</tr>
<tr>
<td>SIF</td>
<td>2.890</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Fig(4): The relationship between the maximum adsorption quantity and inverted temperature for adsorption of Valium on Eggshells in both SGF and SIF.
Table 2: The relationship between the maximum adsorption quantity, inverted temperature and Thermodynamic values for adsorption of Valium on eggshells.

<table>
<thead>
<tr>
<th></th>
<th>T(K)</th>
<th>1000/T (K-1)</th>
<th>Xm</th>
<th>Log Xm</th>
</tr>
</thead>
<tbody>
<tr>
<td>In SGF</td>
<td>300</td>
<td>3.333</td>
<td>5</td>
<td>0.698</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>3.225</td>
<td>5.125</td>
<td>0.709</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>3.125</td>
<td>5.5</td>
<td>0.740</td>
</tr>
<tr>
<td>In SIF</td>
<td>300</td>
<td>3.333</td>
<td>5.5</td>
<td>0.7403</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>3.225</td>
<td>3</td>
<td>0.477</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>3.125</td>
<td>2.5</td>
<td>0.397</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T(K)</th>
<th>1000/T (K-1)</th>
<th>ΔG˚ kJ.mol-1</th>
<th>ΔH˚ kJ.mol-1</th>
<th>ΔS˚ kJ.mol-1.k-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>In SGF</td>
<td>300</td>
<td>3.333</td>
<td>4.467</td>
<td>-0.038</td>
<td>0.0148</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>3.225</td>
<td>4.509</td>
<td>-</td>
<td>0.0145</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>3.125</td>
<td>4.324</td>
<td>-</td>
<td>0.0135</td>
</tr>
<tr>
<td>In SIF</td>
<td>300</td>
<td>3.333</td>
<td>4.054</td>
<td>-0.031</td>
<td>-0.013</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>3.225</td>
<td>6.595</td>
<td>-</td>
<td>-0.021</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>3.125</td>
<td>7.382</td>
<td>-</td>
<td>-0.023</td>
</tr>
</tbody>
</table>

Fig(4)-displays the Van’t Hoff plot for the adsorption of drug from aqueous solutions at different pH. Table 5 shows negative ΔH˚ values in SIF which indicates the exothermic nature of drug adsorption, in contrast with the value in SGF which is endothermic process. This finding was consistent with the results obtained earlier where the drug uptake increased and or decreased with increasing solution temperature depending on pH solutions. Senthilkumaar, et. al(23) have reported that the increase in adsorption uptake with increase in temperature was due to the possibility of an increase in the kinetic energy of the adsorbate molecules.

The positive value of free energy change shows a non-spontaneous adsorption process at the range of temperatures(24). On the other hand, the negative ΔG˚ values give indication about the spontaneous nature of adsorption process which indicates that better adsorption is obtained at low temperature. The negative ΔS˚ values suggest the decrease in adsorbate concentration in solid–solution interface.

Conclusion

Currently, adsorption has been regarded as an effective technology method for the removal of soluble heavy molecules from aqueous solution. The present results in this study showed that eggshells powder was a promising safe low-cost adsorbent to be used in reducing the effect of drug abused from aqueous solutions. Equilibrium data has revealed that the drug adsorption depends on the pH solution as well as the ambient temperature. Drug adsorption was highly temperature dependent. Equilibrium data fitted well to Freundlich
isotherm in SIF solution, which suggests heterogeneity in the sorption sites. kinetics model of the adsorption process was found to follow the PSO in simulated gastric fluid at pH =1.2, while in simulated intestinal fluid at pH =6.8 was fitted to PFO kinetic equations.

**Conflict of Interest:** There is no any Conflict of Interest

**Ethical Clearance:** Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

**Source of Funding:** Self funding.

**References**


Housing for Elderly in Nursing Home on the Level of Satisfaction in the work Area of Social Service of Surabaya

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Abstract

A large number of elderly suffer degenerative brain function decline with the characteristics of progressive loss of memory function and other cognitive abilities. The number is increasing in almost several. The purpose of this study was to look at the effect of housing for the elderly in Nursing Home on the level of satisfaction of the elderly in the Social Service area of Surabaya.

The research design used was “Pre-Experimental” design. The variable in this study was the intervention variable that was the housing for the elderly in the Nursing Home and the output variable that was the satisfaction of the elderly. The population was all the elderly in the work area of the Surabaya Social Service, with the criteria for the age limit of the elderly 60 years and over, with a probability sampling technique. The statistical test used was the Wilcoxon Signed Rank Test.

The results of the study obtained were divided into before and after the housing. Before the housing treatment, there were 133 respondents (88.6%) who were at good satisfaction level and there were 17 respondents (11.4%) who were at very good satisfaction levels. After the housing treatment, the results showed that there were 107 respondents (71.3%) who were at good satisfaction level and there were 43 respondents (28.7%) who were at very good satisfaction level. In addition, the results of the Wilcoxon sign rank statistical test was 0.00. It indicated that there was an effect of housing for the elderly on their level of satisfaction.

Keywords: Elderly, Nursing home, satisfaction

Introduction

A large number of elderly suffer degenerative brain function decline with the characteristics of progressive loss of memory function and other cognitive abilities. The number is increasing in almost several countries. Elderly people tend to experience physical decline and emotional increase, so in terms of architecture, they are certainly different from humans when they were young. According to the Law of the Republic of Indonesia Number 13 of 1998 concerning Elderly Welfare (Undang-undang Nomor 4 Tahun 1965 tentang Pemberian Bantuan Penghidupan Orang Jompo), efforts to improve the welfare of the elderly are still limited to the provision of efforts referred to in Law Number 4 of 1965 concerning the Provision of Livelihood for the Elderly, which is currently found to be inadequate then the development of elderly problems. Therefore, those who have experience, expertise and wisdom need to be given the opportunity to play a role in development. Based on some observations conducted, there are problems at the Nursing Home that make the elderly feel uncomfortable. Therefore, several studies were conducted to obtain the design criteria for the Werdha Nursing Home.

The elderly in 2000 amounted to 7.28% and is projected to increase to 11.34% in 2020, while the population with dementia in 2030 is around 75.6 million and will increase 3 times in 2050 (135.5 million). It shows a fairly high increase which requires immediate treatment and prevention. Meanwhile, around the world, the elderly with dementia reaches almost 47.5 million, from which 58% live in the countries that have low or medium-income per capita (Vega SFD, et al, DOI Number: 10.37506/ijfmt.v15i1.13568

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Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1

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including in Indonesia. Furthermore, Korean government provides subsidies for families who adopt the elderly to be treated at home.²

Broadly the changes experienced by the elderly are divided into 3: physical, psychological and cognitive changes. Physical changes include changes in the sensory system, musculoskeletal system, cardiovascular system, and respiration as well as changes in metabolism; psychological changes include experiencing anxiety, loneliness, and depression; while cognitive changes experienced by the elderly are in the form of decreased memory, IQ (Intelligence Quotient), learning ability (learning), comprehension ability (comprehension), problem-solving, decision making, wisdom, performance, and motivation. As a result, the changes experienced by the elderly cause various disorders, and will have an impact on their satisfaction and quality of life.³ Oliver defines satisfaction as the level of one’s feelings after comparing the performance or the results that he feels with his expectations. The level of satisfaction is a function of the difference between perceived performance and expectations. Individual perceptions of life in society in the context of existing cultural and value systems are related to goals, expectations, standards, and concerns and become a very broad concept that is influenced by individual physical conditions, psychological, level of independence, and the relationship of individuals with the environment. It is referred to as quality of life (World Health Organization Quality of Life (WHOQOL)).⁴ Quality of life is an important indicator to assess health care interventions in terms of prevention and treatment. Low quality of life of the elderly is a result of various diseases that have an impact on decreased productivity and they can not carry out daily activities normally in terms of physical, psychological or mental, social and spiritual, a burden for the family both socially and economically, decreased mental capacity, change in social roles, senility. Depression in the elderly is influenced by several factors namely age, sex, physical ailment, economic, spiritual, insomnia and level of independence.⁵

Panti Werdha (nursing home) is one of the solutions to entrust elderly parents to be cared for and socialize with their peers. Some of them sometimes want to be alone since they do not want to bother their family or relatives and want to spend their old age in peace. Panti Werdha itself, in general, is considered negative by Indonesians. This negative view is compounded by the current condition of the Panti Werdha which is still unfit for habitation without paying too much attention to the comfort and safety. The natural and fostered human environment has a major influence on feelings, behavior, general health problems, and productivity. Humans respond consciously and unconsciously to their homes and workplaces. When a person’s sense of comfort is fulfilled, he will usually respond positively to his environment and people are more receptive to space and content if they provide comfort.⁶ It can be concluded that the environment, both natural and fostered environment more or less bring influence to humans. A comfortable environment will also have a positive impact on the psychological inhabitants. Therefore, in architectural design, an understanding of the characteristics of its inhabitants is needed so that a built environment (architecture) that is suitable for its residents is produced. It also can have a positive impact and create the desired behavior. Likewise, the Nursing Home, by understanding the characteristics of the elderly, is expected to create residential buildings suitable for the elderly. If housing for the elderly in the institution is established and managed effectively, it will be able to increase satisfaction both for patients and for caregivers (nurses). It can also support good development for the cognitive and physical function of patients. A research conducted in 2014 proves that elderly adult patients who do home care are reported to have high levels of life satisfaction and low levels of loneliness and stress levels, compared to those who do not do home care. To maintain quality of life and improve prognosis, care is needed that involves family or home care that is ongoing and uninterrupted from hospital care.⁷

Method

The research design used was “Pre-Experiment” which was one form of research design which intended to uncover a causal relationship from the effect of housing for the elderly in nursing home on the level of their satisfaction. The population in this study was all elderly in the work area of the Surabaya Social Service. The sample of this study was the elderly in the work area of the Surabaya Social Service. Probability sampling technique was simple random sampling. The intervention variable was housing for the elderly in the
nursing home and the output variable was the satisfaction of the elderly. The instrument in this study included; the intervention variable used instrument for housing for the elderly, while the output variable used the instrument for the level of satisfaction of the elderly. This research was conducted in the work area of the Surabaya Social Service. The research was carried out on July 1-20, 2019. The data were then processed and tested using the Wilcoxon Signed-Rank with a significance level of $p \leq 0.05$ (SPSS program).

Ethical issues to guarantee the rights of respondents in conducting this research included: the consent sheet that was given to the subject; the researcher did not include the subject’s name; the confidentiality of the information provided by the subject was guaranteed by the researcher.

**Results**

1. Characteristics of Respondents by Age

Table 1 shows that the respondents were mostly in the seniors category between 60-75 years old with the total of 82 elderly (54.6%), while the rest were in the old age category between 75-90 years with the total of 68 elderly (45.4%).

2. Characteristics of Respondents by Gender

Table 2 shows that there were 79 male respondents (52.6%) and 71 female respondents (47.4%).

3. Characteristics of Respondents Based on Length of Stay

Table 3 shows that from 150 respondents, 28 respondents (18.6%) had lived for 1 year, 63 respondents (42%) had lived for 2 years, and 59 respondents (39.4%) had lived for 3 years or more.
4. Characteristics of Respondents Based on Frequency of Family Visits

<table>
<thead>
<tr>
<th>No</th>
<th>Frequency of visits</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Frequent (&gt; 1 time in 1 month)</td>
<td>16</td>
<td>10.6%</td>
</tr>
<tr>
<td>2</td>
<td>Rare (1 time in 2 months)</td>
<td>42</td>
<td>28%</td>
</tr>
<tr>
<td>3</td>
<td>Never</td>
<td>92</td>
<td>42.28%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>150</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 4 shows that of the 150 respondents found that 16 respondents (10.6%) were frequently visited by their families, 42 respondents (28%) were rarely visited by families, and 92 respondents (61.4%) were never visited by families.

1. Elderly Satisfaction Level before treatment

<table>
<thead>
<tr>
<th>No</th>
<th>Satisfaction Level</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poor</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>Enough</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>Good</td>
<td>133</td>
<td>88.6%</td>
</tr>
<tr>
<td>4</td>
<td>Very good</td>
<td>17</td>
<td>11.4%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>150</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 5 shows that of the 150 respondents, 133 respondents (88.6%) were in the good satisfaction category and 17 respondents were in the very good satisfaction category (11.4%). Details of each aspect of the questionnaire can be seen in the appendix.
2. Elderly Satisfaction Level after treatment

Table 6 shows that out of 150 respondents, 107 respondents (71.3%) were in the good satisfaction category and 43 respondents were in the very good satisfaction category (28.7%). Details of each aspect of the questionnaire can be seen in the appendix.

3. Effect of housing for the elderly in the nursing home on their level of satisfaction

Table 7 shows that a significance value of 0.00 means that there is an effect of housing for the elderly in nursing home on their level of satisfaction.

Discussion

The researcher designed this study to find out the effect of housing for elderly in nursing home on their level of satisfaction in Surabaya Social Service Work Area in accordance with the research objectives. This sub-chapter will discuss the following issues:

1. Elderly Satisfaction Level before treatment

Table 5.5 shows that out of 150 respondents, 133 respondents (88.6%) were in the good satisfaction category and 17 respondents were in the very good satisfaction category (11.4%). Details of each aspect of the questionnaire can be seen in the appendix.

These results indicate that the level of satisfaction of the elderly before getting the treatment in the very good satisfaction category is 88.6%. The facilities or infrastructure available at the Panti Werdha Jambangan are quite adequate, for instance, there are bedrooms per room for 6-8 elderly which are distinguished between men and women. The bathroom is also equipped in each room, and each bathroom has a handle for the elderly. Therefore, it seems that the results were similar with the results before getting the treatment. However, if the results were observed in more detail, it was found that each respondent experienced an increase in the number even though the interval was good satisfaction.
Criteria that can be done to create a home layout for the elderly:

1. Reducing high-risk furniture
2. Additional handrails (toilets, stairs, corridors)
3. Bathroom/toilet seats
4. Good lighting
5. Non-slip/non-slippery floor (carpet and mat stick to the floor)
6. Bright colors
7. Location of the bedroom (downstairs, close to the bathroom)
8. Enlarge the space to walk
9. Pictures of joint activities are posted
10. Family rooms such as recreation rooms are equipped with entertainment facilities.
11. Avoid stacks of decorative objects or statues or objects that can block their space from standing.
12. Avoid stairs
13. The park is equipped with reforestation and therapy support

Conclusion

1. Level of satisfaction of the elderly before treatment in both categories at the Surabaya Social Service
2. The level of satisfaction of the elderly before treatment in both categories at the Surabaya Social Service
3. There is an influence of housing for the elderly with the level of their satisfaction in the Social Service of Surabaya

Confict of Interest : nil

Etichal Clearence : This Research has been approved by STIKES HANGTUAH SURABAYA

Source of Fund : STIKES HANGTUAH SURABAYA

Reference

Public Health in Africa. 2019 Jan 2;10.


The Correlation of DSD Teeth Outline Ratios to Q-Sort Smile Assessment and Smile Mesh Analysis

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Abstract

Smile beauty is considered the main goal in many cosmetic or orthodontic dental treatments. However many objective and sujectives methods were developed to evaluate smile attractiveness depending on many variables. The purpose of this study was to develop a new method based on digital smile design teeth outlines ratios for smile esthetic evaluation and to correlate this method with Q-sort assessment method and smile mesh variables. Frontal posed smile photograph of 48 individuals were subjected to smile mesh measurements, digital smile design (DSD) and Q-sort assessment method. The areas of DSD teeth outlines were measured using image J software in addition to the teeth areas lie in the outlines to calculate the ratio of each individual tooth area to its outline area and the total teeth areas to total outlines area.

The results showed a significant correlation between the new method and Q-sort asessment method, additionally there was a significant difference in individual and total teeth outlines ratios between the pleasant and unpleasant smile groups.

Conclusions It was found that Digital smile design teeth outline ratios method could be considered as a valuable tool for evaluation of smile esthetics.

Key words: Digital smile design, Q-sort, Smile mesh, Tooth outline.

Introduction

One of the main objectives of any cosmetic dental treatment is to obtain a beautiful smile. the pleasant smile necessitate a harmonious and balanced relationship among the lip framework, the gingival scaffold and the teeth¹.

Peck and Peck’s (²) introduced a smile classification based on previous studies about facial expressions and concluded that smile can be either spontaneous or posed, additionally Ackerman et al. (³) classified the smile into posed or social smile and the enjoyment or unopposed smile.

Many authors have referred the unstrained social smile as a reliable reference for measurement and characterization of the smile (⁴, ⁵).

It is necessary to quantify the smile characteristics which considered a distinctive facial feature to help orthodontists and cosmetic dentists not to judge it wholly subjectively due to the absence of a morphometric quantifying tool. Many experimental and clinical studies were performed to identify the esthetic quality of the pleasant smile achieved by cosmetic or orthodontic treatment based on many potential characteristics such as gingival display, incisor protrusion, smile arc and buccal corridors which can greatly influence the esthetic perception of persons (⁶).
Additionally many diagnostic tools that aid in aesthetic diagnosis and prognosis and used for objective smile evaluation such as Objective Grading System (OGS), Diagram of Facial Aesthetic References (DFAR), smile mesh and Digital smile design (DSD). The smile mesh was developed by Ackerman and Ackerman to test the reproducibility and reliability of the posed smile, it comprised five horizontal lines and seven vertical lines superimposed on the posed smile image. This analysis showed that the smiles were reproducible, also it showed high correlation coefficient and high intrarater and interrater reliability between repeated measures (3, 5).

Digital smile design (DSD) planning permit the esthetic evaluation of the intra and extra-oral digital photographs to establish an even gingival contour and proper dental alignment. This final dental outline provides greater predictability to the final result and assisting as a guide to fabricate the diagnostic wax-up and mock-up (7).

**Materials and Method**

Approval for the study was obtained from the scientific committee of orthodontic department in Kufa University faculty of dentistry. The recruited subjects were 48 dental students (28 females and 20 males) to satisfy the Q-sort method design. The sample power was 0.80 as determined by G-power software with respect to correlation tests (Type I error = .05) 5. All 48 subjects included in the study had many characteristics such as: age ranging from 18 - 24 years ; no missing or malformed teeth ; Pleasing faces ; normal occlusion ; no history of previous orthodontic treatment. Additionally many exclusion criteria were considered such as : gummy smile ; canting in the maxillary occlusal plane ; craniofacial anomalies ; history of orthognathic surgery/ cosmetic treatment ; Obvious facial asymmetry (8).

Three photographs were required for smile analysis as follows: full face with a wide smile, full face at rest, and retracted view of the full maxillary arch with teeth apart (9).

Photos were taken by digital camera (Nikon D7100, Japan) with Macro lens (105mm, Sigma), camera settings and patient positioning were performed as mentioned by Vachiramon et al, 2007 (10). While scale measurement and dental photography guidelines were followed as mentioned by Soni et al (11). The subjects were instructed to smile before photographs taking. Ackerman et al. demonstrated the reproducibility of the posed smile obtained from the static photograph 3.

The Smile Mesh parameters were measured via image J software as described by Schabel et al (5) : Maximum incisor exposure ; Upper lip drape ; Lower lip to incisor ; Inter labial gap ; Inter canine width ; Smile width and Smile index.

Then the standardized photos were subjected to digital smile design as describes by Coachman and Calamita (12) and Choachman et al (13). After creating the tooth out line for each of the 8 anterior teeth the image j software used again to measure the area of each tooth outline separately and the total teeth outlines area. Additionally the area of each tooth (of the eight anterior teeth) that lies inside the tooth outline was measured separately in addition to the total teeth area. The ratio of each tooth area to its outline area was calculated in addition to the ratio of total teeth area to the total teeth outlines area. The ratio of individual tooth or total teeth represent the degree of fitness to ideal tooth or teeth outlines and to what extent the tooth or teeth form and position close or far from the ideal smile design.

For Q-sort assessment, the attractiveness of the 48 images was ranked by the panelists (25 orthodontists) from 0-8 according to the method developed by Stephenson which is based on various subjective criteria (14). The average of cut points between attractive and unattractive smiles was calculated to generate the overall demarcation between unattractive and attractive smile images (15). However it was 3.2 in the present study. The samples that have scores less than 3.2 were considered unattractive while those that have 3.2 or above were considered as an attractive smiles.

**Statistical Analysis**

Descriptive statistics included mean and standard deviation. The correlations among variables were examined by Pearson correlation Coefficient. Independent sample t test was used to compare means of all variables. The level of significance for all statistical tests was set at 0.05. All statistical tests were performed with a software program (SPSS version 12.0).
Results

Descriptive statistics of smile mesh variables, DSD ratios and Q-sort scores are shown in table 1. Pearson correlation didn’t show any significant correlation between DSD teeth outlines total ratio and smile mesh variables, while there were a significant correlation with Q-sort assessment (table 2).

After obtaining the average of cut points for Q-sort assessments of the panelists which was 3.2 the total sample was divided into two groups unattractive smile group (<3.2) and attractive smile group (≥ 3.2). An independent samples t test between attractive and unattractive smile groups was done (table 3), for smile mesh variables only upper lip drap and smile width showed a significant difference between the two groups. While for DSD outlines ratios all variables showed a significant differences between the two groups except the tooth 14.

Table 1: Descriptive Statistics of all Q-sort assessment, smile mesh variables and DSD teeth outlines ratios

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>maximum incisor exposure</td>
<td>48</td>
<td>5.00</td>
<td>13.20</td>
<td>9.3</td>
<td>1.7</td>
</tr>
<tr>
<td>upper lip drap</td>
<td>48</td>
<td>-6.00</td>
<td>5.20</td>
<td>-0.021</td>
<td>2.4</td>
</tr>
<tr>
<td>lower lip to incisor</td>
<td>48</td>
<td>0.70</td>
<td>10.50</td>
<td>4.4</td>
<td>2.2</td>
</tr>
<tr>
<td>interlabial gap</td>
<td>48</td>
<td>7.00</td>
<td>21.30</td>
<td>14.34</td>
<td>3.52</td>
</tr>
<tr>
<td>inter canine width</td>
<td>48</td>
<td>32.80</td>
<td>56.90</td>
<td>41.76</td>
<td>4.75</td>
</tr>
<tr>
<td>smile width</td>
<td>48</td>
<td>57.30</td>
<td>93.60</td>
<td>72</td>
<td>8.57</td>
</tr>
<tr>
<td>smile index</td>
<td>48</td>
<td>3.40</td>
<td>8.30</td>
<td>5.3</td>
<td>1.34</td>
</tr>
<tr>
<td>14 ratio 1</td>
<td>48</td>
<td>0.00</td>
<td>0.83</td>
<td>0.48</td>
<td>0.18</td>
</tr>
<tr>
<td>13 ratio 2</td>
<td>48</td>
<td>0.36</td>
<td>0.91</td>
<td>0.71</td>
<td>0.15</td>
</tr>
<tr>
<td>12 ratio 3</td>
<td>48</td>
<td>0.37</td>
<td>0.96</td>
<td>0.72</td>
<td>0.12</td>
</tr>
<tr>
<td>11 ratio 4</td>
<td>48</td>
<td>0.42</td>
<td>0.88</td>
<td>0.72</td>
<td>0.11</td>
</tr>
<tr>
<td>21 ratio 5</td>
<td>48</td>
<td>0.39</td>
<td>0.92</td>
<td>0.72</td>
<td>0.11</td>
</tr>
<tr>
<td>22 ratio 6</td>
<td>48</td>
<td>0.38</td>
<td>0.90</td>
<td>0.67</td>
<td>0.13</td>
</tr>
<tr>
<td>23 ratio 7</td>
<td>48</td>
<td>0.13</td>
<td>0.93</td>
<td>0.64</td>
<td>0.18</td>
</tr>
<tr>
<td>24 ratio 8</td>
<td>48</td>
<td>0.00</td>
<td>0.77</td>
<td>0.42</td>
<td>0.21</td>
</tr>
<tr>
<td>total ratio</td>
<td>48</td>
<td>0.45</td>
<td>0.80</td>
<td>0.67</td>
<td>0.09</td>
</tr>
<tr>
<td>Q-sort assessment</td>
<td>48</td>
<td>1.30</td>
<td>7.10</td>
<td>4.0</td>
<td>1.43</td>
</tr>
</tbody>
</table>

1 Ratio of tooth area of maxillary right first premolar lies inside tooth outline to tooth outline area in DSD.

2 Ratio of tooth area of maxillary right canine lies inside tooth outline to tooth outline area in DSD.

3 Ratio of tooth area of maxillary right lateral incisor lies inside tooth outline to tooth outline area in DSD.
4 Ratio of tooth area of maxillary right central incisor lies inside tooth outline to tooth outline area in DSD.
5 Ratio of tooth area of maxillary left central incisor lies inside tooth outline to tooth outline area in DSD.
6 Ratio of tooth area of maxillary left lateral incisor lies inside tooth outline to tooth outline area in DSD.
7 Ratio of tooth area of maxillary left canine lies inside tooth outline to tooth outline area in DSD.
8 Ratio of tooth area of maxillary left first premolar lies inside tooth outline to tooth outline area in DSD.

Table 2: The significant correlation of total teeth outlines ratio with other variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Minimum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>maximum incisor exposure</td>
<td>48</td>
<td>5.00</td>
<td>0.017</td>
</tr>
<tr>
<td>upper lip drap</td>
<td>48</td>
<td>-6.00</td>
<td>0.000</td>
</tr>
<tr>
<td>lower lip to incisor</td>
<td>48</td>
<td>0.70</td>
<td>0.000</td>
</tr>
<tr>
<td>interlabial gap</td>
<td>48</td>
<td>7.00</td>
<td>0.000</td>
</tr>
<tr>
<td>inter canine width</td>
<td>48</td>
<td>32.80</td>
<td>0.000</td>
</tr>
<tr>
<td>smile width</td>
<td>48</td>
<td>57.30</td>
<td>0.000</td>
</tr>
<tr>
<td>smile index</td>
<td>48</td>
<td>3.40</td>
<td>0.000</td>
</tr>
<tr>
<td>14 ratio</td>
<td>48</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>13 ratio</td>
<td>48</td>
<td>0.36</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3: Independent samples t test between attractive and unattractive smile groups

<table>
<thead>
<tr>
<th>variables</th>
<th>Unpleasant</th>
<th>Pleasant</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper lip drap</td>
<td>-1.60</td>
<td>0.36</td>
<td>0.018</td>
</tr>
<tr>
<td>smile width</td>
<td>75.90</td>
<td>70.38</td>
<td>0.019</td>
</tr>
<tr>
<td>13 ratio</td>
<td>0.62</td>
<td>0.75</td>
<td>0.009</td>
</tr>
<tr>
<td>12 ratio</td>
<td>0.63</td>
<td>0.76</td>
<td>0.000</td>
</tr>
<tr>
<td>11 ratio</td>
<td>0.65</td>
<td>0.75</td>
<td>0.001</td>
</tr>
<tr>
<td>21 ratio</td>
<td>0.68</td>
<td>0.74</td>
<td>0.013</td>
</tr>
<tr>
<td>22 ratio</td>
<td>0.62</td>
<td>0.68</td>
<td>0.035</td>
</tr>
<tr>
<td>23 ratio</td>
<td>0.56</td>
<td>0.67</td>
<td>0.011</td>
</tr>
<tr>
<td>24 ratio</td>
<td>0.34</td>
<td>0.45</td>
<td>0.025</td>
</tr>
<tr>
<td>total ratio</td>
<td>0.60</td>
<td>0.70</td>
<td>0.000</td>
</tr>
<tr>
<td>Q-sort assessment</td>
<td>2.34</td>
<td>4.69</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Discussion

This study was conducted to evaluate the correlation among a new method for smile evaluation using DSD teeth outlines ratios, Q-sort assessment and smile mesh variables. Additionally to compare the variables of these three methods between pleasant and unpleasant smiles.

For DSD analysis there was a significant positive correlation between Q-sort assessment and with most individual teeth outlines ratios and total ratio. However this indicates that high individual teeth outline ratios results in high total ratio which reflect the degree of fitness of the patients teeth to the planned ideal teeth outline according to the guidelines and this ratio can reflect how close or far the patients smile from ideal, unfortunately no previous studies were conducted on DSD to identify a discrepancy index for smile evaluation and analysis; however the reason for significant correlation of individual and total teeth outlines ratios could be explained by that dental, skeletal or soft tissue discrepancies in vertical and/or horizontal dimensions such as asymmetries in the dental arch might play a role in smile attractiveness from visual inspection as mentioned by Schabel et al (16). While for smile mesh variables there was no significant correlation with the total outlines ratio. However this may be due to that these variables are mostly vertical linear measurements and even for horizontal measurements it is difficult to detect asymmetry with this method.

Additionally despite McNamara et al (17) focusing on the role of the lips in the evaluation of the smile, aligned teeth could attract the attention of raters more than the lips, features (16), however this finding could be supported by another one who reported that a greater number of teeth displayed during smiling can give more attractive smile in comparison to fewer teeth (18). Additionally deviation in incisor angulation, incisal plane asymmetry and canting and which were considered as the most noticeable criteria in smile attractiveness evaluation (19, 20) that could affect teeth outlines ratios more than smile mesh variables.

The direct comparison between attractive and unattractive smile groups failed to find any significant differences between the 2 groups of subjects in smile mesh variables except for upper lip drape and smile width this was in disagreement with Schabel et al and McNamara et al (16, 17), but when Schabel et al compared the 11 most attractive smiles with the 11 most unattractive smiles they found that the maxillary incisors to lower lip and the smile index were significantly different, this could be attributed to many factors like the age of individuals which were ranging from 18-25 in the present study compared to 12-20 years in Schabel et al study, also the patients in this study were not treated orthodontically while in Schabel et al study they were finished their orthodontic treatment and finally the comparison in the present study done between all the two groups scores not between the 11 most attractive and 11 most unattractive photos. On the other hand there was a significant difference for all DSD ratios between attractive and unattractive smile groups.

Conclusions

Teeth outlines ratios were significantly correlated Q-sort assessment of smile esthetics. Teeth outlines ratios were significantly increased in pleasant smile group. Teeth outline ratios could be considered as a valuable tool for evaluation of smile esthetics.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References


Pulmonary Artery Hypertension in Acyanotic Congenital Heart Disease Underwent Transcatheter Closure at Dr. Soetomo Hospital

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Abstract

Background: Pulmonary artery hypertension (PAH) is common complication of congenital heart disease. Echocardiography before and after transcatheter closure procedure is needed for further evaluation.

Objective: To evaluate the PAH before and after transcatheter closure procedure by echocardiography at Dr. Soetomo Hospital.

Methods: Medical record patients with acyanotic congenital heart disease with PAH and already done transcatheter closure procedure in 2010 – 2014 were reviewed. Data taken were demographic, clinical, and echocardiography. Tricuspid regurgitation pressure gradient (TRPG) was evaluated. Statistical analysis using t test comparative study; P<0.05 was considered significant.

Results: There were 46 patients underwent transcatheter closure, ASD closure 22/46, VSD closure 16/46, PDA closure 8/46 patients. Ten patients with PAH (10/46), 4/10 ASD, 4/10 PDA and 2/10 VSD. Boys were 6/10, median age was 60 (range 4-144 months). Median TRPG before procedure was 32.4 (range 25-43 mmHg). Median TRPG after procedure was 21.5 (range 15-26.9 mmHg). There was significant decreased in PAH after transcatheter closure procedure (P=0.01).

Conclusion: Transcatheter closure procedure in acyanotic congenital heart disease was important for decreasing the pressure gradient of PAH.

Keywords: Pulmonary artery hypertension, acyanotic congenital heart disease, tricuspid regurgitation, transcatheter closure

Background

Pulmonary arterial hypertension (PAH) is a common complication of congenital heart disease (CHD), particularly in patients with left-to-right (systemic-to-pulmonary) shunts. Persistent exposure of the pulmonary vasculature to increased blood flow and pressure may result in vascular remodelling and dysfunction. This leads to increased pulmonary vascular resistance (PVR) and, ultimately, to reversal of the shunt and development of Eisenmenger’s syndrome.¹ The estimated prevalence of CHD is approximately six to 10 per 1,000 live births and 4–15% of patients with CHD will go on to develop PAH²⁻³. The development of PAH in patients with CHD is associated with increased mortality and high morbidity, reflected in a substantial increase in health service utilisation. While successful early closure of a cardiac defect prevents the development of PAH, have led to a marked decrease in the prevalence of PAH-CHD in western countries, the number of patients with CHD surviving into adulthood has increased.⁴⁻⁵

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The interventional approach has become increasingly preferred for the treatment of many congenital heart defects (CHDs), including atrial septal defects (ASDs), ventricular septal defects (VSDs), and patent ductus arteriosus (PDAs). Transcatheter closure of congenital heart defects has been widely accepted as a preferred treatment; however, the high cost of these devices limits their clinical application in some countries. Echocardiography plays crucial role in the safety and efficacy assessment of transcatheter treatment. The advantage of TEE guidance and assistance during the closure procedure is shorter fluoroscopic time. The transcatheter closure of heart defect is nowadays accepted as a safe and effective treatment modality. In the process of selection of eligible defects play crucial role, the closure procedure should be performed by TEE guidance and follow-up of closure results by monitoring of TTE.

This study was designed to evaluate PAH before and after transcatheter closure procedure by echocardiography at Dr. Soetomo Hospital.

Methods and Materials

The cross-sectional study was conducted during January 2010–December 2014 at Division of Cardiology Department of Pediatrics Dr. Soetomo Hospital. Patients were selected based on inclusion and exclusion criteria. The inclusion criteria were 1) patients with acyanotic congenital heart disease 2) underwent transcatheter procedure in Dr. Soetomo Hospital. Patients were excluded if 1) Had multiple congenital anomaly, 2) had a severe infection or severe condition. There were 46 patients acyanotic heart disease diagnosed by echocardiography and underwent the transcatheter closure procedure. The characteristic data including age, sex, chief complaint, physical examination laboratory, chest X-ray and data from echocardiography were collected from medical records. The data taken from echocardiography were include type of congenital defect, size, pressure gradient, and ejection fraction.

A total of ten patients got PAH as complication of congenital heart disease, they were compared pressure gradient before and after transcatheter closure procedure. Statistical Analysis used t-test comparative study, with P<0.05 considered significant. All analyses were conducted in SPSS version 21.

Result

During the study period, 46 patients acyanotic congenital heart disease were included in this study. The basic characteristic in this study (table 1) found that sex was dominated by Male (58%), the age of children mostly in >5 (45%) years old and the median age was 5 years. Dyspnea was the most common main complain in our study 73.9%, and followed by recurrent cough 17.3% and fatigue were 8.08%. The atrial septal defect were affected on 22 (47%) patients, ventricular septal defects were 16 (34%) patients and patent ductus arteriosus 8 (17%) patients. The patients were found mostly with malnutrition status (52%), however found well nourish patients (40%), and severe malnutrition (8%). Ten patients had complication pulmonary artery hypertension (PAH). Four patient had PAH with ASD, 4 patient PAH with PDA and two patients less with VSD. Typed Degree of Tricuspid Regurgitation were dominated with mild (90%) and less of moderate (10%).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(N=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: Female</td>
<td>19 (41%)</td>
</tr>
<tr>
<td>Male</td>
<td>27 (58%)</td>
</tr>
<tr>
<td>Age (yr): &lt;1</td>
<td>10 (21%)</td>
</tr>
<tr>
<td>≥ 1-5</td>
<td>15 (32%)</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>21 (45%)</td>
</tr>
</tbody>
</table>

Table 1. Baseline Characteristics of acyanotic congenital heart disease underwent transcatheter closure
Table 2 show median degree of Tricuspid Regurgitation Pressure Gradient (TRPG) before procedure was 32.4 (range 25-43 mmHg). Median TRPG after procedure was 21.5 (range 15-26.9 mmHg). We used t test comparative study to compare degree of TRPG. There was significant decreased in PAH after transcatheter closure procedure ($P=0.01$) with $P<0.05$ considered significant.

Table 2. Median of Tricuspid Regurgitation Pressure Gradient before and after transcatheter procedure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before transcatheter closure</th>
<th>After transcatheter closure</th>
<th>$P$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median TRPG</td>
<td>32.4 (25-43)</td>
<td>21.5 (15-26.9)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*p significant $<0.05$

**Discussion**

In this study, the patients who got acyanotic congenital heart disease during January 2010 – Desember 2014 at Division of Cardiology Department of Pediatrics Dr. Soetomo Hospital were 46 patients and most of all was male (58%). Based on study on Kathmandu university of Nepal in 2008 showed that presentation of acyanotic congenital heart disease was dominated with male with male : female ratio = 1.5:1 8. The number of age patients was most above than 5 years old (45%) and ASD was the most presentation in our study despite of PDA and VSD. It could be caused by our participants is from tertiary care center as a main referral from all patients in east region of Indonesia. Most of the patients came to our hospital not from earlier age. The former studies are usually passive, in that diagnosis is made in large regional high-quality pediatric cardiology center but relies on referral of patients from local doctors. Thus, if a local physician is comfortable with the management of tiny VSD that patient might not be referred to a center and so not be countered. The relative frequency of acyanotic congenital heart disease shows from the
highest was VSD, ASD, and PDA respectively 35%, 35%, 33%. In research reported that VSD, PDA and ASD Most lesions in infants born alive are 34%, 23.7% and 10.8%, while most lesions on stillbirth is ASD (17.7%), VSD (14.6%), and TOF (13.8%). In addition, some lesion with subtle physical findings, such as ASD might not be detected until they appear in adult life. 9,10

In our study the patients were found mostly with malnutrition status (52%), however found well nourish patients (40%), and severe malnutrition (8%). Patients with increasing blood flow to the lung and had pulmonary hypertension will suffered from malnutrition and stunted growth associated with hypoxia patients. This situation will facilitate infection that worsen the condition. Anorexia, inadequate nutrition, hypoxemia tissue, hipermetabolik status, acidemia, and cation imbalances, decreased peripheral blood flow, chronic cardiac decompensation, malabsorption and protein loss, tract infections recurrent respiratory, hormonal factors, and genetic will eventually lead to malnutrition condition 11

Dyspneu was the mostly main complain in our study (73.9%) recurrent cough (17.3) and the less was fatigue (8.08%). Most frequently, patients congenital heart disease complain of progressive shortness of breath. For example a studies have shown a reduction in maximum oxygen consumption in the unrepaired ASD population because of the inherent inefficiency of a continuously preload-reduced LV in combination with a volume overload in the pulmonary circulation. After repair of the ASD, exercise capacity improves within days to weeks. 12

In our study, complication of PAH associated with congenital heart disease was (21.7%). In the French National Registry of PAH, PAH-CHD was the second most commonly associated form of PAH (after connective tissue disease-associated PAH). Data from European registry studies give the overall prevalence of PAH in adult patients with CHD as 4–28% and the prevalence of Eisenmenger’s syndrome as ,1–6% 13,14

Median degree of Tricuspid Regurgitation Pressure Gradient (TRPG) before procedure in our study was 32.4 (range 25-43 mmHg). Median TRPG after procedure was 21.5 (range 15-26.9 mmHg). There was significant decreased in PAH after transcatheter closure procedure. Correction of an underlying congenital heart defect in infancy can prevent the development of PAH-CHD; however, a proportion of patients with left-to-right shunts are not recognized until later in their life, when they already have changes to the pulmonary vasculature and increased PVR. In those patients with increased PAP and Qp, but with a PVR within normal limits or only slightly raised, pulmonary vascular changes are likely to be minimal and the patient may benefit from surgery. Conversely, those patients with high PAP and high PVR are likely to have extensive changes to the pulmonary vasculature and corrective surgery is contraindicated. There remains, therefore, a population of patients with medium-to-large defects and moderate increases in PVR in whom the extent of pulmonary vascular changes and their potential to be reversed are unknown and so, in whom, the benefits or otherwise of corrective surgery are unclear. Improvements in the diagnosis of CHD and its surgical and medical management have led to a significant increase in the number of patients surviving into adulthood. The best therapy for PAH-CHD remains prevention through a “timely” repair of the defect. 15 This study had limitation that not enough sample and participants so need further longitudinal and multicenter study.

**Conclusion**

In our conclusion, children with acyanotic heart disease commonly associated with complication of PAH. Transcatheter closure procedure in acyanotic congenital heart disease was important for decreasing the pressure gradient of PAH. But this study need further research to identifying, assessing and accurately deciding on management strategies.

**Conflict of Interest :** None declared.

**Source of Funding :** The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

**Ethical Clearance :** Approved by researched ethical committee Dr. Soetomo General Hospital Surabaya.

**References**


Correlation between Elevated Serum Progesterone in the Day of Hcg Injection, Metaphase II Oocytes and Their Impact on The Success of ICSI

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Abstract

Objective: In this study we tried to understand the effect of progesterone level at the time of ovulation trigger on intracytoplasmic sperm injection (ICSI) outcome. We expected identifying cut off values for serum progesterone level the day of hCG injection (P-hCG) & P-hCG per mature oocyte ratio (P-hCG / MII), as predictive tests of ICSI success. 

Methods: 200 women 18-40 years old and attending IVF unit of Kasr el Aini hospital for management of infertility were included in the study. They were scheduled for ICSI after controlled ovarian induction with GnRH- agonist protocol. Inclusion criteria were tubal factor of infertility, unexplained infertility, polycystic ovarian syndrome and BMI ≤ 30 kg/m2. Exclusion criteria were severe male factor infertility, hyperprolactinemic patients, high basal FSH > 11 IU/L, frozen embryo transfer cycles, uterine anomalies or synechia. Primary outcome parameter included correlation of serum progesterone on day HCG administration, number of MII oocytes and calculated progesterone/ MII oocyte ratio with success of ICSI.

Results: 193 cases underwent embryo transfer, 7 cases cancelled. Clinical pregnancy was reported in 52 (27.9%) cases. Higher P-hCG was observed in cases who didn’t get pregnant compared to those who got pregnant (p= 0.01) with cut off value of 1 ng/ml correlated well with the clinical pregnancy rate after ICSI with 61% sensitivity and 59% specificity (p=0.004). A significantly lower P-hCG/MII ratio was found in the pregnant women group compared to that found in the non-pregnant group (p=0.001) and cut off value of 0.18 correlated best with clinical pregnancy after ICSI with sensitivity 70.5% and specificity 61.2% (p<0.001).

Conclusion: We demonstrated negative correlation between P-hCG and P-hCG/MII and clinical pregnancy rate after ICSI.

Keywords · ICSI; Infertility; P-hCG/MII oocytes ratio;

Introduction

Futile infertility treatment is associated with psychological and financial burdens. Thus, several studies were conducted to search for an accurate marker for ICSI success (eg. D3 FSH/LH ratio, serum progesterone level on the day of hCG injection (P-hCG), number of mature oocytes retrieved …etc.) which would allow suitable selection of stimulation protocols and appropriate counseling before attending the stressful and expensive course of IVF. This is particularly important for patients belonging to lower and middle socioeconomic classes and cannot afford multiple cycles of IVF.

Outcome of IVF depends on number and quality of oocytes and endometrial receptivity. Moderate increase in serum progesterone in the peripheral circulation occurs in most super-ovulated cycles on the day of HCG injection. Ubaldi et al (1996) concluded the higher FSH exposure in the IVF cycles and the subsequent higher FSH-induced LH receptivity in granulosa cells might be one of the factors inducing premature luteinization but this didn’t cause adverse effects on IVF outcome.
Bourgain & Devroey (2003) concluded that “the endometrium of IVF cycles showed premature secretory changes in post-ovulatory phase of IVF cycles followed by dys-synchronous glandular and stromal differentiation in the mid-luteal phase.” This suggests a profound modification of luteal endometrial in stimulated cycles.  

The time of maximal endometrial receptivity “the implantation window” is characterized by the expression of endometrial products, among which pinopodes, integrins and leukemia inhibitory factors are most described. Premature expression of pinopodes and integrins are observed with precocious luteal transformation and increased progesterone level following ovarian stimulation. 

There is debate about origin and significance of elevated serum progesterone in stimulated cycles. It has been believed that increased LH in late follicular phase cause increased progesterone and the use of gonadotropin-releasing hormone (GnRH) agonists and antagonists to prevent the rise of LH and premature luteinization was tried. Results of this premature elevation of serum progesterone on IVF outcome is controversial. 

Several authors did not find any negative effect of this on IVF outcome. Other authors reported that pregnancy rate has been inversely related to P-hCG. 

In this study we tried to understand the effect of progesterone level at the time of ovulation trigger on intracytoplasmic sperm injection (ICSI) outcome. We expected identifying cut off values for P-hCG & P-hCG per mature oocyte ratio (P-hCG /MII), as a better predictive tests of ICSI success.

**Materials and Methods**

Following ethical approval of scientific committee of OB/GYN department, Kasr Al-Aini hospital, Cairo University, Egypt, 200 women aged 18-40 years old and attending the IVF unit of Kasr el Aini hospital (2015-2018) for management of infertility were included in the study after obtaining informed consent. All women were scheduled for ICSI after controlled ovarian induction. **Inclusion criteria** were normal serum prolactin level, tubal factor of infertility, unexplained infertility, polycystic ovarian syndrome and BMI ≤ 30 kg/m2. **Exclusion criteria** were severe male factor infertility, hyperprolactinemic patients, high basal FSH > 11 IU/L, frozen embryo transfer cycles, uterine Anomalies or synechia, repeated implantation failure in ICSI (more than 3 failed trials), thyroid dysfunction, history of ovarian surgery. 

All women included in the study were subjected to careful history taking and general and local examination, AFC & AMH were recorded and BMI calculation was done. All patients were tested for basal serum FSH, LH, & E2 levels on the third day of a spontaneous cycle. Patients was subjected to testing of basal serum progesterone on day 3 of the down regulated cycle (E2<50 pg/ml) before stimulation with HMG and then repeated again on day of hCG administration to determine possible deleterious effect arising from elevated LH levels on pregnancy rates. Serum FSH, LH, E2 and Progesterone were measured using chemiluminescent enzyme immunoassay (Immulate 2000 Siemens Medical Solutions Diagnostic). Endometrial thickness on the day of hCG injection was determined using Sonoace x4 ultrasound machine (Samsung Medison Co., Ltd. Seoul, South Korea), duration of stimulation, total dose of gonadotropins used, number of retrieved, fertilized oocytes, embryo grading, number of transferred good quality embryos, & cancellation rates per cycle were determined. 

The standard long GnRH agonist protocol was used for patients with predicted normal response based on clinical & hormonal profile; 1 mg of leuprolide acetate daily s.c injection (Lucrin ®; Abbott, Hoofddorp, The Netherlands) was applied from the mid luteal phase onward till the day of HCG injection. Gonadotropins in the form of HMG (Merional ®, IBSA, Institut Biochimique SA, Lugano, Switzerland) was given IM from the 2nd day of menstruation, The starting dose range from 150 to 450 IU depending on the basal FSH level, AFC, Patient’s age and BMI. In all protocols, stimulation was monitored by Tran-vaginal ultrasonography and serial E2 measurements starting from day 7 of the cycle and the gonadotropin dose was adjusted individually according to follicular response. After the development of at least three leading follicles≥18 mm, 10,000 unit of HCG (Choriomon, IBSA, Institut Biochimique SA) was given IM, and transvaginal ultrasound-guided oocyte retrieval was performed 36 hours later. Progesterone pessaries
400 mg twice daily (Cyclogest 400 mg ® Actavis plc. Dublin, Ireland) was given as a luteal support starting from the day of embryo transfer and continued for 16 days after. Pregnancy was defined as the occurrence of a positive β-HCG >10 IU on day 12 after embryo transfer and a second higher value 2 days later, followed by ultrasonography confirmation of cardiac activity at 6 weeks gestation (for clinical pregnancy).

**Primary outcome parameter included** Correlation of P-hCG and calculated P-hCG/ MII ratio with Success of ICSI and ROC Analysis to determine accuracy of these tests in predicting clinical pregnancy in ICSI Cycles. **Secondary outcome parameters included** cut off levels for P-hCG and P-hCG/MII in predicting successful IVF outcome (clinical pregnancy).

**Statistical analysis:** Data were statistically described in terms of mean ± standard deviation ±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing 2 groups of normally distributed data and Mann Whitney *U* test for independent samples for comparing not-normal data. For comparing categorical data, Chi-square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity, and specificity. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

**Results**

Two hundreds patients meeting the inclusion criteria included in the present study divided into 2 groups, 193 cases underwent embryo transfer, 7 cases cancelled (all in group A (FSH/LH ≥3) due to failure to yield mature follicle for fertilization. Clinical pregnancy was reported in 52 (27.9%) cases. Primary infertility was the type of infertility in 148 patients (74%) and it was secondary in 52 patients (26%). The indications for ART treatment included male factor infertility (40.5% n=81), PCO (24.5% n=49), unexplained infertility (16% n=32) and tubal factor infertility (19% n=38).

**Demographic characteristics:** The mean age was 29.83±4.43 years old, the mean BMI was 26.5±2.9 kg/m², the mean duration of infertility was 4.63±2.59, the mean FSH was 6.55±1.54 mIU/ml, the mean LH was 4.77±1.5 mIU/ml and the mean FSH/LH ratio was 1.57±0.8. The mean E2 was 36.13±10.1 pg/ml, the mean AMH was 3.9±3.15 ng/ml, the mean AFC was 13.7±SD5.9. The mean basal serum progesterone was 0.76±0.25 ng/ml while the mean serum progesterone in the day of hCG injection was 1.4±0.9. Table 1 shows ovarian stimulation parameter.

<table>
<thead>
<tr>
<th>Table (1): Parameters of Ovarian stimulation and ICSI cycle.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>Stimulation Duration/day</td>
</tr>
<tr>
<td>Total Gonadotropin dose/IU</td>
</tr>
<tr>
<td>Trigger day Endometrial Thickness/ml</td>
</tr>
<tr>
<td>No of Retrieved oocytes (196 cases)</td>
</tr>
<tr>
<td>No of MII oocytes (196 cases)</td>
</tr>
<tr>
<td>P-hCG/MII (193 cases)</td>
</tr>
<tr>
<td>Fertilized oocytes (196 cases)</td>
</tr>
<tr>
<td>Fertilization ratio (193 cases)</td>
</tr>
<tr>
<td>No of Transferred embryos (193 cases)</td>
</tr>
</tbody>
</table>


\[ P-\text{hCG/MII} = \text{serum progesterone level the day of hCG injection per mature oocyte ratio} \]

- Studying Clinical pregnancy:

Table (2) studies the difference in the selected parameters between patients who got pregnant to those who didn’t. There was only a significant statistical difference in \( P-\text{hCG} (p=0.01) \) and \( P-\text{hCG/MII ratio} (p=0.001) \), while FSH/LH ratio, basal progesterone, and endometrial thickness on the day of hCG trigger were all non-significant (\( p=0.05 \), \( p=0.7 \), and \( p=0.4 \) respectively).

Table (2): The impact of study parameters on clinical pregnancy rate.

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Clinical pregnancy YES (n=54)</th>
<th>Clinical pregnancy NO (n=139)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH/LH</td>
<td>1.37±0.59</td>
<td>1.59±0.8</td>
<td>0.05</td>
</tr>
<tr>
<td>P basal</td>
<td>0.77±0.24</td>
<td>0.75±0.26</td>
<td>0.71</td>
</tr>
<tr>
<td>P-hCG</td>
<td>1.23±0.76</td>
<td>1.57±0.98</td>
<td>0.01*</td>
</tr>
<tr>
<td>P-hCG/MII</td>
<td>0.19±0.17</td>
<td>0.3±0.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Trigger day End. Thickness</td>
<td>11.83±2</td>
<td>11.56±2.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD.

* = statistically significant. \( P \) basal = serum progesterone before stimulation, \( P-\text{hCG} \) = serum progesterone in day of hCG injection, End = endometrial

- Diagnostic accuracy of the studied parameters:

ROC analysis was performed to determine cut off values for FSH/LH ratio, P-hCG/MII ratio and P-hCG that may correlate with clinical pregnancy. The following table (3) and figures shows the results with specificity and sensitivity for each. The cut off value of \( P-\text{hCG} \) (Figure 1) was 1 ng/ml with 61% sensitivity and 59% specificity (\( p=0.004 \)), while the best cut off value for \( P-\text{hCG/MII ratio} \) (Figure2) was 0.18 with sensitivity 70.5% and specificity 61.2% (\( p<0.001 \)).

Table (3): Cut-off values for predicting IVF success (clinical pregnancy?).

<table>
<thead>
<tr>
<th></th>
<th>Cut Off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-hCG</td>
<td>1</td>
<td>61%</td>
<td>59%</td>
</tr>
<tr>
<td>P-hCG/MII</td>
<td>0.18</td>
<td>70.5%</td>
<td>61.2%</td>
</tr>
</tbody>
</table>

\( P-\text{hCG} = \text{serum progesterone level the day of hCG injection, } P-\text{hCG/MII} = P-\text{hCG per mature oocyte ratio.} \)
Figure (1): ROC curve for P-hCG as a predictor of clinical pregnancy after ICSI in our study patients (AUC =0.632 and p=0.004). At a P-hCG threshold of 1 ng/ml sensitivity was 61% and specificity 59%.

Figure (2): ROC curve for P-hCG/MII ratio as a predictor of clinical pregnancy after ICSI in our study patients (AUC =0.71 and p<0.001). At a P-hCG/MII ratio threshold of 0.18 sensitivity was 70.5% and specificity 61.2%.
Discussion

In our study, we tried to evaluate the role of serum progesterone in day of hCG injection in predicting ICSI outcome. We were also able to determine the optimal cut off value for P/MII oocyte ratio which might give us better prediction of ICSI success.

Higher P-hCG was observed in cases who didn’t get pregnant compared to those who got pregnant (1.57±0.98 vs 1.23±0.76 respectively, P= 0.01) and cut off value for P-hCG of 1 ng/ml correlated well with the clinical pregnancy rate after ICSI with 61% sensitivity and 59% specificity (p=0.004).

There has been an ongoing debate regarding the impact of preovulatory P on IVF outcome. Its clinical influence has been highly controversial for many years.

Schoolcraft et al., 1991, showed that the premature progesterone elevation was related to a lower pregnancy rate when using GnRH agonist for pituitary suppression. Progesterone levels more than 0.5 ng/ ml were associated with a lower rate of pregnancy (20%) compared with less than 0.5ng/ml (54%). These results showed that ovarian hyperstimulation might cause advanced luteinization and an adverse cycle outcome even with low-LH levels.12

Venetis et al. (2007) published an early systematic review and meta-analysis and revealed a lower pregnancy rate in patients with high serum progesterone, but the difference was not statistically significant.13 However, Kolibianakis et al., (2012) in a more recent analysis found that women undergoing ovarian hyperstimulation using GnRH antagonists and gonadotropins, progesterone rise on the day of hCG injection is significantly associated with a lower possibility of clinical pregnancy.14

In an analysis on the outcomes of 2,566 cases after their first IVF/ICSI cycles managed with long or short protocols of GnRH agonists it was found that a premature progesterone elevation negatively correlated with live birth rate in fresh embryo-transfer (ET) cycles. However, live birth rates in frozen-thawed ET cycles showed no significant difference between case with or without progesterone rise implying that progesterone rise in stimulated cycles may have deleterious effects on endometrial receptivity.15 In a big analysis of more than 4,000 cycles, it was found that clinical pregnancy rates following IVF/ICSI cycles were inversely associated with serum progesterone levels on the day of hCG injection, regardles the GnRH analogue used. In particular, patients with serum progesterone levels < 1.5ng/ml had significantly higher clinical pregnancy rates than those with progesterone levels >1.5ng/ml.16

Although many studies have showed an adverse relation between elevated progesterone levels and IVF/ICSI pregnancy outcomes, the precise endocrinological mechanism is not clear. It has been theorized that progesterone in the late follicular phase might influence endometrial development which may lead to an asynchrony between the endometrium and the implanted embryo.17 Li et al in 2011 from micro RNA and microarray analysis of endometrium suggested dissimilar endometrial changes in patients with high progesterone levels on the day of hCG injection, and had poor pregnancy rates.8

On the other hand, several authors failed to demonstrate any negative effect of progesterone rise on IVF outcome.18,19,20,21 Using a previously described breakpoint in serum progesterone concentration of 0.9 ng/ ml in an earlier study, it was found that an elevated serum progesterone level on the day of hCG does not adversely affect the quality of oocytes or resulting embryos. The results suggest that the pregnancy rate in the elevated serum progesterone group is at least equal to the observed rate in the low progesterone group.22

A later investigation demonstrated that in the presence of an adequate response to ovarian stimulation progesterone levels > 0.9 ng/ml were not associated with lower pregnancy rates, indicating that good embryo quality may compensate for the adverse endometrial effects of elevated progesterone. However, when the response to ovarian stimulation was weak, premature progesterone elevation led to drastically reduced pregnancy rates.19,20

Another study found that there is no association between late follicular serum progesterone concentration on the day of hCG and the biochemical and clinical pregnancy rates obtained after ovarian stimulation for IVF/ICSI. Instead, a strong significant association was found between the number of follicles/oocytes and serum progesterone concentration, suggesting that each individual follicle contributes to the collective concentration observed in the circulation. Paradoxically,
the highest pregnancy rate in the study was found in the group of patients who had the highest late follicular progesterone concentrations (i.e.47nmol/l) and thus developed many follicles.\(^{18}\)

Some authors concluded that serum progesterone cutoff value that negatively affect the outcome of IVF should be considered according to the ovarian response; which can be “normally higher” (about 1.75 ng / ml) in the cycles with ovarian hyper responsiveness.\(^{23,24}\)

Based on these findings, It seems that elevated serum progesterone concentrations are a frequent event in GnRH analogue treated cycles and this elevation seems to be directly related to the number of oocytes obtained. According to various studies because the main sources of progesterone are follicles, stimulated by FSH, the level of progesterone produced per mature oocyte would be a better predictor for IVF outcome.\(^4\)

Thus, we tried to evaluate the impact of the ratio between P-hCG and the number of mature oocytes retrieved on ICSI outcome. We found that significantly lower P-hCG/MII ratio (mean=0.19±0.17) was found in the pregnant women group compared to that found in the non-pregnant group (mean=0.3±0.3) (P=0.001). Cut off value of 0.18 correlated best with clinical pregnancy after ICSI with sensitivity 70.5% and specificity 61.2% (p<0.001).

**Conclusion**

Our study showed significant difference in P-hCG and P-hCG/MII oocyte ratio among patients with clinical pregnancy and those who didn’t get pregnant, however these tests have low diagnostic accuracy in predicting clinical pregnancy.

Using the above data, larger groups of patients can be analyzed to confirm our findings in larger studies. This study is also limited by its non-randomized design; future larger randomized trials are required to evaluate the role of P- hCG/MII oocyte ratio as a valuable prognostic tool in IVF over P levels alone.

**Conflict of Interest:** The authors have no conflicts of interest.

**Source of Funding:** Personal fund.

**Ethical Committee Approval:** Ethically approved by the department.

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Nutritional Status, Dust Exposure and Risk Factors for Acute Respiratory Infections for Workers in Industrial Estates

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Abstract

Acute Respiratory Infections (ARI) are a health problem that has not been handled properly in Indonesia, every year the incidence of acute respiratory infections is still high, which can be fatal, namely death. Several factors cause acute respiratory infections such as nutritional status, dust exposure, length of exposure to dust, and years of service for workers in industries who have a high risk of acute respiratory tract infection problems. This study aims to determine the risk factors for acute respiratory infections in the industry. This study uses a cross-sectional approach with a sample of 89 respondents, where the results will be analyzed using the chi-square test. The result of this study is that there is a relationship between nutritional status, dusty workspace, length of exposure to dust, and length of work with the incidence of acute respiratory infections in industrial workers. Based on the results of this study, it is hoped that workers and the industry will pay attention to the use of PPE to minimize dust exposure to workers in the industry.

Keywords: nutritional status, dust, acute respiratory infections

Introduction

Industry in Indonesia is currently experiencing very rapid development, where industrial growth in Indonesia is currently experiencing an increase of 7% from the previous year. The industry is one that has become a mainstay and has received special attention from the Indonesian government. Along with the development of the industry not only has a positive impact but often has a negative impact caused by negligence from both managers and workers. Work accidents and occupational diseases are problems that have not been handled properly until now1,2.

Every workplace always contains various potential hazards that can affect the health of workers or cause occupational diseases. One of the potential hazards in the workplace that can cause health problems is the potential for chemical hazards, especially those used in the production process3. These potential hazards can enter or affect the body of the workforce through inhalation (through breathing), ingestion (through the mouth to the digestive tract, and skin contact (through the skin). The effect of potential chemical hazards on the workforce depends on the type of chemical or contaminant, the form of potential hazards (dust, gas, vapor, and smoke), toxicity, and entry into the body4.

Dust produced from industrial processes is a particle that is easily inhaled by workers so that it can irritate the respiratory tract. The textile-producing industry is one of the industries that produce exposure to dust particles from the production process, this is one of the triggers for the occurrence of acute respiratory infections (ARI). The incidence of ISPA in Indonesia is one of the diseases that many people experience which can cause death4,5. A person

DOI Number: 10.37506/ijfmt.v15i1.13574
who lives in a rural area is attacked by ARI in one year on average three times and for urban areas, it can be up to six times. For people who work with exposure to dust particles have a much greater risk of getting an ARI\textsuperscript{7,8}.

ARI in industrial areas occurs due to several factors, one of which is the extrinsic factor. The first is the state of the material being inhaled (gas, dust, vapor). Dust that enters through inhalation can cause extensive fibrosis in the lungs and is antigenic. Other extrinsic factors are smoking behavior, length of exposure, behavior in using personal protective equipment (PPE), especially those that protect the respiratory system and exercise\textsuperscript{9}. Intrinsic factors from within humans, especially those related to the lung defense system, both anatomically and physiologically, history of illness, gender, body mass index\textsuperscript{10,11}.

**Material and Method**

This study uses a cross-sectional study. The population in this study were 89 workers who were taken by random sampling, using data analysis, namely chi-square. This research was conducted in an industry in Indonesia, West Java Province. The variables studied were nutritional status, workspace, dust exposure, and years of service to the incidence of ARI among workers in the industry.

**Findings**

Table 1. Analysis of Risk Factors for ARI

<table>
<thead>
<tr>
<th>Bivariate</th>
<th>Not ARI</th>
<th>ARI</th>
<th>Total</th>
<th>P-Value</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Nutritional Status</td>
<td>19 (61.3%)</td>
<td>12 (38.7%)</td>
<td>31 (100%)</td>
<td>0.001</td>
<td>0.895 (0.367 (-2,183)</td>
</tr>
<tr>
<td>Abnormal Nutritional Status</td>
<td>34 (58.6%)</td>
<td>24 (41.4%)</td>
<td>58 (100%)</td>
<td>0.000</td>
<td>0.782 (0.378 (-1,850)</td>
</tr>
<tr>
<td>Dusty Workspace</td>
<td>28 (50.9%)</td>
<td>27 (49.1%)</td>
<td>55 (100%)</td>
<td>0.000</td>
<td>0.793 (0.388 (-1,860)</td>
</tr>
<tr>
<td>Dustless Workplace</td>
<td>25 (73.5%)</td>
<td>19 (38%)</td>
<td>54 (100%)</td>
<td>0.000</td>
<td>0.793 (0.388 (-1,860)</td>
</tr>
<tr>
<td>Duration of Dust Exposure &lt; 8 jam/hari</td>
<td>22 (56.4%)</td>
<td>17 (43.6%)</td>
<td>39 (100%)</td>
<td>0.000</td>
<td>0.793 (0.388 (-1,860)</td>
</tr>
<tr>
<td>Duration of Dust Exposure ≥ 8 jam/hari</td>
<td>31 (62%)</td>
<td>19 (38%)</td>
<td>50 (100%)</td>
<td>0.043</td>
<td>1.310 (0.436 (-3,932)</td>
</tr>
<tr>
<td>Years of service &lt; 5 tahun</td>
<td>11 (64.7%)</td>
<td>6 (35.3%)</td>
<td>17 (100%)</td>
<td>0.043</td>
<td>1.310 (0.436 (-3,932)</td>
</tr>
<tr>
<td>Years of service ≥ 5 tahun</td>
<td>42 (58.3%)</td>
<td>30 (41.7%)</td>
<td>72 (100%)</td>
<td>0.043</td>
<td>1.310 (0.436 (-3,932)</td>
</tr>
</tbody>
</table>
Discussion

Nutritional Status

The results of the study found 12 workers with normal nutritional status (38.7%) suffered from ARI, while 24 (41.4%) workers with abnormal nutritional status suffered from ARI. The analysis showed that the p-value was 0.001 < α 0.05, which means there was a relationship between nutritional status and the incidence of ARI.

If a person lacks food intake, it will cause the immune system to become weak, making it easier for that person to get infectious diseases. In the case of malnutrition, individuals will be more susceptible to infection due to decreased immunity to invading pathogens12.

Dusty Workspace

The results of the study found that 19 (38%) of workers with dust-free workspaces suffered from ARI, while 27 (49.1%) of workers with dusty workspaces suffered from ARI. The results of the analysis show that the p-value is 0.000 < α 0.05, which means that there is a relationship between dusty workspace and the incidence of ARI.

Dust is a material that is often referred to as particles floating in the air with a size of 1 micron to 500 microns. In the case of air pollution both inside and outside the building, dust is often used as an indicator of pollution. Used to show the level of danger both to the environment and to occupational health and safety13–15.

Exposure to dust can cause acute respiratory problems, one of which is industrial products that can pollute the air such as coal dust, cement, cotton, asbestos, chemical substances, toxic gases, dust in rice mills (organic dust) and others. Various factors influence the emergence of disease or airway disorders due to dust16,17.

Duration of Dust Exposure

The results of the study found that 17 workers exposed to dust <8 hours/day (43.6%) suffered from ARI, while 19 workers who were exposed to dust ≥ 8 hours/day had ARI (38%). The results of the analysis show that the p-value is 0.000 < α 0.05, which means that there is a relationship between dust exposure and the incidence of ARI.

The longer a person works, the higher the risk level for pulmonary function disorders. Besides, the length of service determines the length of time a person is exposed to the risk factors of exposure to dust, so the greater the length of time a person is exposed, the greater the risk of developing lung disease13,18,19.

The threshold value (TLV) for dust content is the standard of work environment factors that are recommended in the workplace so that workers can still receive them without causing illness or health problems, in daily work for a time not exceeding 8 hours a day or 40 hours a week (Permenakertrans RI No.13 of 2011). For dust particles, it has been stipulated in the Minister of Manpower and Transmigration Regulation No. PER 13/MEN/X/2011 concerning the threshold value for physical and chemical factors in the air of the working environment is that TLV dust levels should not exceed 3.0 mg/m³. The threshold value for dust that only interferes with work enjoyment is 10mg/m³. TLV of dust concentration in ambient air in Indonesia is also regulated in the Decree of the Minister of Health of the Republic of Indonesia Number 1405/MENKES/SK/XI/2002 concerning health requirements for office and industrial work environments, amounting to 10mg/m³ for an average measurement time of 8 hours2,4,8.

Years of Service

The results of the study found that 6 (35.3%) of workers with a working period of <5 years suffered from ARI, while workers who had a work period of ≥ 5 years suffered from ARI as much as 30 (41.7%). The results of the analysis show that the p-value is 0.043 <α 0.05, which means that there is a relationship between years of service and the incidence of ARI.

The effect of dust on the respiratory tract has shown that dust levels are associated with the incidence of respiratory symptoms, especially coughs. In the respiratory tract, the dust that settles to accumulate mucosal edema on the walls of the respiratory tract, causing respiratory constriction20,21.

The effect of industrial dust levels on the work environment must be watched out for because the dust in the environment is in the air and is always...
inhaled by workers every day\textsuperscript{9}. If workers who work in an environment with high dust concentrations for a long time will have the risk of developing respiratory problems, especially with workers who have worked for more than 5 years in a work environment with high concentrated respiratory dust\textsuperscript{22} The length of exposure in a day is one of the risk factors in the occurrence of pulmonary function disorders in workers. That the longer the working period of a person, the higher the risk level in the occurrence of pulmonary function disorders\textsuperscript{1,21,23}.

Conclusion

The results showed that nutritional status, dusty workspace, years of work, duration of dust exposure were related to the incidence of ARI among industrial workers.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of Ibn Khaldun University.

All subjects were fully informed about the procedures and objectives of this study each subject before the study signed an informed consent form.

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17. Palmer K, McNeilssl-Love R, Poole J. Inflammatory responses to the occupational inhalation of metal


Expression of FOXP1 and p53 in Reactive Lymphoid Lesion and B-cell Non-Hodgkin Lymphoma, Large Cell Type

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Abstract
Lymphoproliferative lesions that have morphology between benign and malignant are difficult to diagnose even with immunohistochemical and clonality testing. The correct diagnosis is necessary for the prompt treatment. These lesions can also serve as instructive models of lymphomagenesis. FOXP1 plays an important role in B-cell development, has a potential oncogene in B-cell Non-Hodgkin lymphoma, and p53 protein has a crucial role in the regulation of cell cycle, DNA repair, apoptosis, and senescence tumor suppression activity. In this study, we analyze the role of FOXP1 and p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type. 68 paraffin blocks samples from patients diagnosed as reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type. 68 paraffin blocks samples from patients diagnosed as reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type was sectioned and stained with immunohistochemistry for FOXP1 and p53, and the percentage of nuclear cells showing positive staining were evaluated. Expression of FOXP1 and p53 in B-cell Non-Hodgkin lymphoma, large cell type is higher than in reactive lymphoid hyperplasia with p=0.001 and cutoff point 45%(CI=95%) for FOXP1 and p=0.001 and cutoff point 7.5%(CI=95%) for p53. There is a significant correlation between the expression of FOXP1 and p53 in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type (p=0.001). Our findings suggest that high expression of FOXP1 and p53 in B-cell Non-Hodgkin lymphoma may demonstrate the role of FOXP1 and p53 in lymphomagenesis and these markers may help to distinguish benign and malignant lymphoproliferative lesions.

Keywords: reactive lymphoid hyperplasia, B-cell Non-Hodgkin lymphoma, FOXP1, p53

Introduction
Lymph nodes are major components in the immune system. They react to various stimuli by undergoing reactive changes[1]. Recent research identifies lymphoproliferative lesions that interface between benign lesions and malignancies[2]. Distinguishing the reactive lymph node from a neoplastic lymphoproliferative process is one of the very crucial things in order to avoid harmful treatment for patients who do not need the therapy and vice versa[3]. Detection of clonality in a suspected lymphoproliferative lesion is important in diagnostic criteria[4], such as Bcl2/IGH and CCND1/IGH translocation associated with in situ forms of follicular lymphoma and mantle cell lymphoma, but sometimes clonal populations of B and T lymphocytes have been identified in many reactive or infectious disorders, and many lymphoma- or leukemia-associated translocations have been identified in the peripheral blood of healthy individuals[2].

The aim of this research is to study the role FOXP1 and p53 in lymphomagenesis, so this may help to distinguish borderline lesions between atypical...
lymphoproliferative lesions and malignant lymphoma. FOXP1 has an important role in regulation of B-cell development and maturation\textsuperscript{5}, also known as an oncogene in various types of B-cell Non-Hodgkin lymphoma\textsuperscript{6}, while p53 is the most important molecular marker in malignancy including diffuse large B-cell lymphoma. Loss of normal p53 activity is associated with lymphomagenesis and mediates tumors resistant to chemotherapy\textsuperscript{7}. In this study, the truly benign lesions (reactive lymphoid hyperplasia) and truly malignant lesions (B-cell Non-Hodgkin lymphoma, large cell type) were used to represent the benign lymphoproliferative lesions and malignant lymphoproliferative lesions.

**Materials and Methods**

This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (0040/LOE/301.4.2/VI/2020).

**Research Design and Sample**

This was an analytic observational research with a cross-sectional approach. There were 68 samples, each of 34 formalin-fixed, paraffin-embedded tissues were obtained from patients diagnosed as reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type patients during 2017-2018 in Anatomical Pathology Laboratory, Dr. Soetomo General Hospital, Surabaya, Indonesia.

**Immunohistochemical Staining**

The formalin-fixed, paraffin-embedded tissues were cut into 4 mm sections, deparaffinized with xylol for 5 minutes three times, and rehydrated through graded alcohol. Antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH 6.0) for 10 minutes. The tissue sections were then incubated with monoclonal antibodies for FOXP1 (CMC35032010; dilution 1:400; Cell Marque, Abcam Technology) and p53 (DS-0337-C; dilution 1:100; Diagnostic Bio System) overnight, followed by secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol.

**Evaluation of Immunohistochemical Expression**

All samples were evaluated blindly by 2 observers. This study assessed the expression of FOXP1 and p53 by calculating the percentage in the entire field of view using a light microscope, Olympus (40× magnification)\textsuperscript{8,9}. The percentage of FOXP1 and p53 in the entire field of view was calculated using a semiquantitative method by dividing the number of mature lymphocyte cells in reactive lymphoid hyperplasia and tumor cells in B-cell Non-Hodgkin lymphoma, large cell type that are stained brown in the nuclei by the total population of the sample cells.

**Statistical Analysis**

All statistical analyses were calculated using SPSS v25.0. The comparison of FOXP1 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell types was tested using unpaired T test. The comparison of p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type was tested using Mann Whitney U test. The correlation was analyzed using Spearman test, with a significance level <0.05 (p <0.05).

**Results and Discussion**

The clinicopathological characteristics of the patients are shown in Table 1. FOXP1 was expressed at the nuclei of mature lymphocytes in the reactive hyperplasia and nuclei of tumor cells in Non-Hodgkin lymphoma (Figure 1). A significant difference of FOXP1 expression was found between reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type (\( p = 0.001 \)) (Table 2), which was higher in B-cell Non-Hodgkin Lymphoma, large cell type than in reactive lymphoid hyperplasia, with cutoff point 45\%(CI=95\%). Pearson correlation test showed a significant correlation between FOXP1 expression of reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type (\( r_p = 0.793, p = 0.001 \)). The expression of p53 was expressed at the nuclei of mature lymphocytes in the reactive hyperplasia and nuclei of tumor cells in Non-Hodgkin lymphoma (Figure 2). A significant difference of p53 expression was found (\( p = 0.001 \)) (Table 3) and expression in B-cell Non-Hodgkin lymphoma, large cell type is higher than in reactive lymphoid hyperplasia with cutoff point 7.5\%(CI=95\%). Spearman correlation test showed a significant correlation between p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type (\( r_s = 0.768, p = \)
The expression of FOXP1 and p53 in reactive lymphoid hyperplasia and B-cell Non-Hodgkin Lymphoma, large cell type has a significant positive correlation ($r_s = 0.640$, $p = 0.001$) and shown in Table 4.

Table 1. Clinicopathological characteristics of the patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Reactive Lesion (n) (%)</th>
<th>B-cell NHL (n) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>9 (26.47%)</td>
<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>1 (2.94%)</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>3 (8.82%)</td>
<td>2 (5.88%)</td>
</tr>
<tr>
<td>31-40</td>
<td>3 (8.82%)</td>
<td>3 (8.82%)</td>
</tr>
<tr>
<td>41-50</td>
<td>6 (17.64%)</td>
<td>4 (11.76%)</td>
</tr>
<tr>
<td>51-60</td>
<td>8 (23.53%)</td>
<td>14 (41.17%)</td>
</tr>
<tr>
<td>61-70</td>
<td>4 (11.76%)</td>
<td>8 (23.53%)</td>
</tr>
<tr>
<td>71-80</td>
<td>0</td>
<td>2 (5.88%)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>0</td>
<td>1 (2.94%)</td>
</tr>
<tr>
<td>Mean age</td>
<td>35.5±23.36</td>
<td>53.73±13.60</td>
</tr>
<tr>
<td>Age range</td>
<td>2-70</td>
<td>25-81</td>
</tr>
<tr>
<td>Sex</td>
<td>(n (%))</td>
<td>(n (%))</td>
</tr>
<tr>
<td>Male</td>
<td>26 (76.5%)</td>
<td>22 (64.7%)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (23.5%)</td>
<td>12 (35.3%)</td>
</tr>
<tr>
<td>Location</td>
<td>(n (%))</td>
<td>(n (%))</td>
</tr>
<tr>
<td>Neck</td>
<td>16 (47.06%)</td>
<td>16 (47.06%)</td>
</tr>
<tr>
<td>Mesenterium</td>
<td>6 (17.64%)</td>
<td>0</td>
</tr>
<tr>
<td>Axilla</td>
<td>2 (5.88%)</td>
<td>0</td>
</tr>
<tr>
<td>Submandibule</td>
<td>4 (11.76%)</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>1 (2.94%)</td>
<td>4 (11.76%)</td>
</tr>
<tr>
<td>Intraabdomen</td>
<td>2 (5.88%)</td>
<td>3 (8.82%)</td>
</tr>
<tr>
<td>Inguinal</td>
<td>3 (8.82%)</td>
<td>2 (5.88%)</td>
</tr>
<tr>
<td>Extranodal</td>
<td>0</td>
<td>9 (26.47%)</td>
</tr>
</tbody>
</table>

Table 2. Expression of FOXP1 in Reactive Lymphoid Hyperplasia and B-Cell Non-Hodgkin Lymphoma.

<table>
<thead>
<tr>
<th>Status</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Lesion</td>
<td>44.4</td>
<td>15.99</td>
<td>5</td>
<td>88</td>
<td>0.001</td>
</tr>
<tr>
<td>B-cell NHL</td>
<td>83.6</td>
<td>14.59</td>
<td>38</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

*Unpaired T test applied
Table 3. Expression of p53 in Reactive Lymphoid Hyperplasia and B-Cell Non-Hodgkin Lymphoma.

<table>
<thead>
<tr>
<th>Status</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Lesion</td>
<td>10.61</td>
<td>30.72</td>
<td>0</td>
<td>30</td>
<td>0.001</td>
</tr>
<tr>
<td>B-cell NHL</td>
<td>55.38</td>
<td>30.72</td>
<td>5</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

*#Mann Whitney U test applied
p-value < 0.05, considered as significant

Table 4. Correlation between FOXP1 and p53 expression in Reactive Lymphoid Hyperplasia and B-Cell Non-Hodgkin Lymphoma.

<table>
<thead>
<tr>
<th>FOXP1 expression</th>
<th>p53 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r_s</td>
</tr>
<tr>
<td></td>
<td>0.640</td>
</tr>
</tbody>
</table>

Figure 1. Immunohistochemical expression of FOXP1(brown staining in nuclei) (400× magnification). A,B. Immunohistochemical expression of FOXP1 in reactive lymphoid hyperplasia (A: 30% staining, B: 60% staining). C,D. Immunohistochemical expression of FOXP1 in B-cell Non Hodgkin Lymphoma, large cell type (C: 90% staining, D: 98% staining). Black arrows: FOXP1 expression; bar: 50 μm.
Lymphoproliferative lesions with similar morphologic feature could be a difficult area in histopathology. Therefore, detection of clonality in these cases plays important role to diagnostic criteria. The key characteristic of cancer is the monoclonality of tumor cells, which derivatives of transformed malignant cells, which should be done in the dubious conditions[4]. Ideally, further immunohistochemical examination also needed to determine the subtypes of Non-Hodgkin lymphoma with similar morphology[10], for example, the subtypes of DLBCL are determined by gene expression profiling, which is the gold standard for identifying the GCB and ABC subtypes, but is not routine practice[3].

Our study showed that FOXP1 expression was higher in B-cell Non-Hodgkin lymphoma, large cell type than reactive lymphoid hyperplasia and both of them showed significant correlation. Patzelt et al stated that FOXP1 transcription factors are important in the early development of B-cell. FOXP1 is deregulated through chromosome translocation in mature B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL). Deficiency of FOXP1 in early lymphoid precursors results in the cessation of pro-B cell transitions to pre-B and reduces peripheral matured B-cell. Overexpression of FOXP1 in DLBCL and B-cell suppresses several proapoptotic genes such as Bik, Eaf2, and Hrk, in collaboration with NF-κB activity to support B-cell resistance. It is suspected that high expression of FOXP1 in lymphoma cells prevents cells from undergoing apoptosis. FOXP1 knockdown on DLBCL cells induces an increase in MHC II expression, so that it can have an effect on immunosurveillance tumors during lymphomagenesis[11].

Figure 2. Immunohistochemical expression of p53 (brown staining in nuclei) (400× magnification). A,B. Immunohistochemical expression of p53 in reactive lymphoid hyperplasia (A: 20% staining, B: 30% staining). C,D. Immunohistochemical expression of p53 in B-cell Non Hodgkin Lymphoma, large cell type (C: 90% staining, D: 98% staining). Black arrows: p53 expression; bar: 50 μm.
Expression of p53 also showed higher in B-cell Non-Hodgkin lymphoma, large cell type than reactive lymphoid hyperplasia and both of them are significantly correlated. This result is similar with research by Kanavaros et al. who found that in non-neoplastic conditions, the role of p53 besides inhibiting the cell cycle is also involved in apoptosis. The p53 protein influences the expression of Bcl2 and Bax, which are involved in the regulation of apoptosis. Bcl2 which acts as an antiapoptotic undergoes downregulation, whereas Bax which induces apoptosis experiences upregulation by p53. The proapoptotic effect of Bax arises through performing activity as opposed to Bcl2. Bax also works as a tumor suppressor gene. Several studies have shown that Bcl2 and Bax proteins play a role in lymphoid malignancies[12]. Genetic factors that disrupt DNA repair or apoptosis can increase precancerous risk[13].

The wild type p53 is tightly controlled at the post translational stage. Under physiological conditions, the level of wild p53 is low due to constitutive degradation by E3 ubiquitin ligase MDM2 which is a target of wild type p53 transcription. Conversely tumors that have mutant p53 are typically characterized by a substantial accumulation of p53 protein[14]. The p53 gene mutation and dysregulation of the p53 pathway are important in the pathogenesis of cancers including lymphomas. TP53 dysfunction in lymphoid malignancies can occur at the level of DNA, mRNA, or protein in cis or trans. Many mutant p53 work in a dominant-negative way to inhibit the function of wild-type p53. Single allele mutations are often followed by loss of heterozygosity, which then supports tumor development. The p53 mutation points in lymphoma malignancies occur most often in the p53 DNA-binding domain (DBD). Simultaneously, p53 and inactivation of other genes, for example p21, cause a poor prognostic effect. The combination of p53+/p21-immunophenotype can reflect the p53 mutation with prognostic value[15].

We found that FOXP1 and p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type was significantly correlated. Study by He et al., described the activity of FOXP1 depends on miR34a downregulation, which allows the development of B-cell. Inactivation of miR34a influences the pathway of B-cell development, consistent with the abnormality seen with the expression of FOXP1, p53, and Bcl2 that affect the development of mature B-cell and supports malignant transformation associated with B-cell lymphoma[16]. Networks of p53 through FOXP1 and Bcl2 are connected by miR34a which is a tumor suppressor. The effect of miR34a on FOXP1 which suppresses p53 is potentially oncogenic on post germinal center B-cell. Parallel effects also occur on the role of miR34a as a link between p53 and the oncogenic protein Bcl-2. The effect of p53 on the B-cell developmental pathway is consistent with the abnormalities found in p53 deficiency, namely the increasing number of pre-B cell as well as B-cell, which is also a consequence of the loss of miR34a function[17]. The effect of miR34a on FOXP1 in the form of the mechanism of p53 suppressing tumor cells implies a connection between p53 and FOXP1 through the action of miR34a[16].

Conclusion

In summary, the expression of FOXP1 and p53 in B-cell Non-Hodgkin lymphoma, large cell type, is higher than in reactive lymphoid hyperplasia. This suggest that FOXP1 and p53 have a role in lymphomagenesis and these markers may help to distinguish benign and malignant lymphoproliferative lesions. The suspicion for lymphoid malignancy will be raised if the expression of FOXP1 is above 45% and p53 is above 7.5%. Further investigation in other types of lymphoma such as low-grade lymphoma should be carried out because they have different biology behavior.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: This study supported by the Ministry of Education and Culture of the Republic of Indonesia.

Acknowledgements: We thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (0040/LOE/301.4.2/VI/2020).

References

2011.


The Correlation between Body Mass Index and Lifting Frequency with Low Back Pain Complaints on Rice Transport Workers in Warehouse of Perum BULOG Subdivre Pematangsiantar

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Abstract

Introduction: Rice transport workers are at risk for Low Back Pain (LBP) complaints because their work is lifting heavy loads repeatedly and affected by individual characteristics. This research aimed to analyze the strength of the correlation between body mass index and lifting frequency with LBP complaints on rice transport workers in Perum BULOG Pematangsiantar Subdivre.

Methods: This type of research was an observational research with a cross sectional design. The sampling technique used total populated sampling with 30 respondents. The variables researched included body mass index, lifting frequency, and LBP complaints.

Data were collected by observation, filling out research questionnaires, and Nordic Body Map (NBM). The data analysis used was the Spearman correlation.

Results: The strength of the correlation between body mass index with low back pain complaints had a value of r = 0.203, the strength of the correlation between the lifting frequency with low back pain complaints had a value of r = 0.415.

Conclusion: Body mass index with LBP complaints had a weak and positive correlation. The lifting frequency with LBP complaints had a moderate and positive correlation.

Key words: body mass index, lifting frequency, low back pain complaints

Introduction

Occupational diseases are diseases caused by work or the work environment, including work-related diseases. The work-related disease has several causative agents with occupational factors or the work environment playing a role together with other risk factors. Musculoskeletal disorders are one of the occupational diseases. Musculoskeletal disorders are complaints of parts of the skeletal muscles that are felt by a person ranging from very mild to very painful complaints.

In a survey conducted on workers in the UK, it was found that 498,000 workers had work-related musculoskeletal disorders, and as many as 40% had work-related musculoskeletal disorders on the back. Low Back Pain (LBP) is often reported in jobs that require routine heavy lifting, especially when combined with other stressors, such as unnatural positions (awkward positions) or exposure to whole-body vibrations. Truck
Drivers, nurses, construction workers, cleaners, and warehouse workers are at high risk for back pain due to work. The prevalence of LBP in Indonesia is 18% and increases with age. Low Back Pain is a complaint that can reduce human productivity. Around 50-80% of workers worldwide have experienced low back pain, which has a negative impact on socio-economic conditions by decreasing working day and decreasing productivity.

Factors that can affect the emergence of low back pain include individual factors, namely age, gender, body mass index (BMI), work period, smoking habits, and physical activity, as well as work factors, namely workload, work position, repetition, and duration. Based on the results of research conducted by Mayasari on fishers in the Kangkung Village, Bandar Lampung showed that there is a correlation between the lifting frequency with LBP complaints.

Musculoskeletal disorders in the back generally arise due to manual material handling. Manual handling is any activity of lifting or supporting a load by hand or body strength in lifting, putting, pushing, pulling, carrying, or moving activities.

Badan Urusan Logistik (BULOG) is a state-owned public company engaged in food logistics. Perum BULOG Subdivre Pematangsiantar is assigned to carry out public service activities, plan and develop business activities, especially in the rice sector. Perum BULOG Subdivre Pematangsiantar is located at Asahan Street KM. 3.5, Pematangsiantar, North Sumatra, Indonesia.

Perum BULOG Subdivre Pematangsiantar had two warehouses. The warehouse of Perum BULOG Subdivre Pematangsiantar was used to store commodity items. There were activities carried out manually at the Perum BULOG Subdivre Pematangsiantar, namely the loading and unloading processes. This loading and unloading processes were carried out where the workers place the rice sack on the workers back. The workers will walk towards the truck, arriving at the truck the workers will lower the rice sack by dumping it down on a low pile of rice or asking other workers for help if the rice pile is high, and vice versa when transporting rice from trucks to warehouses. Transport workers take turns carrying out this activity.

There were 30 transport workers at Perum BULOG Subdivre Pematangsiantar and were daily workers. Transport workers worked when there were items in and out, in a week the rice transport workers work for about four days. Rice transport workers start at 8 am and stop working when the unloaded or loaded rice runs out. Transport workers can carry 50 kg of rice sacks with a transport distance of approximately 20 meters.

Transport workers had complaints of pain in the lower back, neck, shoulders, knees, and calves. From observations, it was known that the transport workers’ work position when lifting rice, such as the bent neck, raised arms, slightly bent back, and alternately bent legs. The loading and unloading work was done repeatedly, and this could result in excessive exertion and could cause body aches. Based on the problems explained in the introduction above, the researcher has carried out the strength of the correlation analysis between body mass index and lifting frequency with low back pain complaints on rice transport workers in warehouse of Perum BULOG Subdivre Pematangsiantar.

Materials and Methods

This research was an observational research with a cross-sectional design. This research was conducted on rice transport workers in the warehouse of Perum BULOG Subdivre Pematangsiantar, North Sumatra, Indonesia, in May 2020. The sampling technique in this research used total population sampling and obtained a sample of 30 people.

Measurement of Body Mass Index (BMI) was carried out by filling out a research questionnaire. Before filling out the research questionnaire, respondents were given an explanation of the research conducted and asked to fill out an informed consent if the respondent agreed to take part in the research. Measurement of the lifting frequency was carried out by observation, then categorized into two according to the median data. BMI was categorized into severe underweight (BMI <17), mild underweight (BMI 17-18.4), normal (BMI 18.5-25), mild overweight (BMI 25.1-27.1) and severe overweight (BMI> 27) 7. Then the lifting frequency was categorized into <103 times and ≥ 103 times.
Nordic Body Map (NBM) method was used to assess LBP complaints. NBM has been used to assess LBP complaints in several studies including research conducted by Setyawan with the title of the correlation between work attitudes and transport loads on low back pain (LBP) complaints in fish transport workers (manol) in Muncar Beach Fishing Port (PPP), Banyuwangi District. In addition, NBM was also used by Riningrum in her research titled the influence of work attitude, age, and work period on low back pain complaints, with garment sewing workers of PT. Apac Inti Corpora, Semarang District as respondents. In the research, NBM method used was only on lower back. This research was also used part of NBM which was on lower back part, because this research focused on low back pain.

The data analysis technique in this research was univariate and bivariate analysis. Univariate analysis was conducted to determine the frequency distribution of each variable. Meanwhile, bivariate analysis was carried out to analyze the strength of the correlation between BMI and lifting frequency with LBP complaints using the Spearman correlation test.

Result and Discussion

Body Mass Index

Table 1. Distribution of Transport Workers Based on BMI

<table>
<thead>
<tr>
<th>BMI</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Underweight (BMI 17–18,4)</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Normal (BMI 18,5-25)</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>Mild Overweight (BMI 25,1-27)</td>
<td>7</td>
<td>23,3</td>
</tr>
<tr>
<td>Severe Overweight (BMI&gt;27)</td>
<td>2</td>
<td>6,7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 showed that transport workers’ BMI in Warehouse of Perum BULOG Subdivre Pematangsiantar mostly were in normal category as many as 18 workers with a percentage of 60%.

Lifting Frequency

Table 2. Distribution of Transport Workers Based on Lifting Frequency

<table>
<thead>
<tr>
<th>Lifting Frequency</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;103 Times</td>
<td>14</td>
<td>46,7</td>
</tr>
<tr>
<td>≥103 Times</td>
<td>16</td>
<td>53,3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 showed that most lifting frequency of transport workers in Warehouse of Perum BULOG Subdivre Pematangsiantar was in ≥103 times category amounted to 16 workers with a percentage of 53,3%.
Low Back Pain Complaints

Table 3. Low Back Pain Complaints in Rice Transport Workers

<table>
<thead>
<tr>
<th>Low Back Pain Complaints</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Painless</td>
<td>5</td>
<td>16,7</td>
</tr>
<tr>
<td>Slightly Hurt</td>
<td>10</td>
<td>33,3</td>
</tr>
<tr>
<td>Hurt</td>
<td>11</td>
<td>36,7</td>
</tr>
<tr>
<td>Very Hurt</td>
<td>4</td>
<td>13,3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 showed that LBP complaints in transport workers in Warehouse of PERUM BULOG Subdivre Pematangsiantar mostly were on hurt category as many as 11 workers with a percentage of 36,7%.

Correlation between Body Mass Index with Low Back Pain Complaints

Table 4. Cross Tabulation of BMI with Low Back Pain Complaints

<table>
<thead>
<tr>
<th>BMI</th>
<th>Low Back Pain Complaints</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Painless</td>
<td>Slightly Hurt</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Mild Underweight</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>16,7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16,7</td>
</tr>
<tr>
<td>Mild Overweight</td>
<td>2</td>
<td>28,6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>28,6</td>
</tr>
<tr>
<td>Severe Overweight</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>16,7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13,3</td>
</tr>
</tbody>
</table>

Table 4 showed that most transport workers with normal BMI had slightly hurt LBP complaints as many as six workers (33,3%) and hurt LBP complaints as many as six workers (33,3%). Based on the statistical analysis result with the Spearman Correlation test, it was obtained correlation coefficient value of 0,203 which means that the correlation between BMI of transport workers with LBP complaints had a weak strength of correlation. Based on the direction of correlation, it was obtained that the correlation between transport workers’ BMI with LBP complaints have a positive correlation which means that the higher transport workers’ BMI, then the level of LBP complaints experienced by
workers in Warehouse of Perum BULOG Subdivre Pematangsiantar would increase.

When body weight increases, the spine will be pressured to receive the burdens that weigh so can lead to easily occurred damage and danger to the structure of the spine. One of the areas in the spine most at risk from the effect of obesity is lumbal vertebrae\(^1\).

This was in accordance with the research conducted by Mayasari which states that there is a correlation between BMI with LBP complaints on fisherman in Kangkung Village, Bandar Lampung\(^11\). This research was also in line with research carried out by Maulana which indicates that there is a correlation between BMI with level of pain in patients with Low Back Pain (LBP) in Poliklinik Saraf RSUD Dr. Zainoel Abidin, Banda Aceh\(^10\).

**Correlation between Lifting Frequency with Low Back Pain Complaints**

The following is cross tabulation of data between lifting frequency with low back pain complaints.

<table>
<thead>
<tr>
<th>Lifting Frequency (times)</th>
<th>Low Back Pain Complaints</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Painless</td>
<td>Slightly Hurt</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>&lt;103</td>
<td>5</td>
<td>35.7</td>
</tr>
<tr>
<td>≥103</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Table 5 showed that the majority of transport workers with lifting frequency of ≥103 times that had hurt LBP complaints were as many as seven workers (43.8%). Based on the results of statistical analysis with the Spearman Correlation test, it was obtained correlation coefficient value of 0.415 which means the correlation between lifting frequency with low back pain complaints had a moderate strength of correlation. Based on the direction of correlation, it was obtained that the correlation between lifting frequency with LBP complaints had a positive correlation which means the more transport workers lift weight then LBP complaints experienced by transport workers in Warehouse of Gudang Perum BULOG Subdivre Pematangsiantar would increase.

The movement frequency that is too often will encourage fatigue and muscle tensions in the tendon. Muscle tensions in the tendon can be relieved when there is a rest period used to stretch the muscle. The impact of repetitive movements will increase if the movement is performed in awkward positions with heavy loads repeatedly. The occurrence frequency of posture is related to the number of times repetitive motion in doing work. Muscle complaints happen because muscle receives pressure caused by loads repeatedly without having a chance to relax\(^3\).

This result is in accordance with research conducted by Mayasari which states that there is a correlation between lifting frequency with low back pain complaints in fisherman in Kangung Village, Bandar Lampung\(^11\).
In research done by Coenen states that there is a correlation between lifting frequency with low back pain occurrences.

Constant repetitive movements give cumulative workloads, can lead to a sense of pain in impaired muscles function and other soft tissues. Problems arising from repetitive work or excess muscle pressure include muscle fatigue, changes in tissue density, and tissue tension. Physiological evidence suggests that the level and degree of tissue damage depends on the amount of force, repetition, and duration of exposure.

**Conclusion**

Based on the research result, it can be seen that most rice transport workers in Warehouse of Perum BULOG Subdivre Pematangsiantar had normal body mass index (18.5-25) and lifting frequency of ≥103 times. The majority of rice transport workers in Warehouse of Perum BULOG Subdivre Pematangsiantar had LBP complaints category of hurt.

There is a weak and positive correlation between body mass index with LBP complaints in rice transport workers in Warehouse of Perum BULOG Subdivre Pematangsiantar. There is a moderate and positive correlation between lifting frequency with LBP complaints in rice transport workers in Warehouse of Perum BULOG Subdivre Pematangsiantar.

It is suggested that rice transport workers, specifically who have worked for a long time to do some exercise or muscle stretching before doing any lifting and transport activities for at least 10 minutes because stretching muscles is very good for flexibility of the spinal muscles. The recommended exercise to prevent low back pain complaints is ≥3 times per week. Transport workers was expected to have enough rest and meet their nutritional needs before working.

**Acknowledgments:** Thank you to all parties that are involved in the research, especially rice transport workers in Warehouse of Perum BULOG Subdivre Pematangsiantar who have been willing to become respondent in this research.

**Ethical Clearance:** This research was approved and obtained ethical permission from the Faculty of Dentistry, Airlangga University, Surabaya, Indonesia.

**Source of Funding:** The source of this research costs from self

**Conflict of Interest:** Nil

**References**


Analysis of Genetic Variations And Specific Locus of Banjar Hulu Tribe Through Short Tandem Repeat (STR) Locus Combine DNA Index System (CODIS) in Nuclear DNA

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Abstract

Based on the 2010 Indonesian Population Census, there are 1,300 ethnic categories with various sub-tribal details. Ethnic diversity is important in terms of identification, such as natural disasters, accidents, crime, and the discovery of unidentified corpses that may come from various tribes. Forensic Medicine views genetic diversity and variation as one of the challenges in identification. Genetic variation and kinship in Banjar Hulu tribe have never been done. The aim of the study are identify and analyze the genetic variation of Banjar Hulu tribe through nucleus DNA examination of short tandem repeat (STR) Loci combined DNA index system (CODIS). This study is a descriptive observational study that aims to explain the genetic variation of Banjar Hulu tribe. The design of this study is cross-sectional. This study was conducted in two stages, the first stages is to obtain information on family tree from the study subjects conducted by interview. Then the second stage is to draw blood from the study subjects’ criteria. Blood is drawn from peripheral vein. Then, there will be DNA examination and analysis of genetic variation. Result of the study show THO1 allele 9.3 locus, D3S1358 allele 12 locus, and D16S539 allele 12 locus, D8S1179 allele 12 locus are typical marker for Banjar Hulu tribe. Banjar Hulu tribe have specific loci and alleles.

Keywords: Banjar Hulu tribe, STR, CODIS.

Introduction

Indonesia is a maritime country that has more than 17,000 islands between the Pacific Ocean and the Indian Ocean. Indonesia has 730 regional languages and various tribes, Indonesia is one of the most diverse countries on earth in terms of ethnicity, linguistics and genetics.1-4 Based on the 2010 Indonesian Population Census there are 1,300 ethnic categories with various sub-tribal details.5 Ethnic diversity is distributed on various islands in Indonesia, most of which are found on five major islands, namely Sumatra, Kalimantan, Java, Sulawesi and Papua.6-8 Kalimantan Island is divided into five provinces, one of them is South Kalimantan Province. Most of the population in the South Kalimantan region is the Banjar tribe.9 South Kalimantan has several disaster-prone areas. The disaster consisted of floods, whirlwinds, fires, landslides, and forest fires. Disasters in South Kalimantan are increasing every year and have an impact on society and destruction. Disasters can leave corpses that may be intact, half intact, decomposed, separated in fragments, burned to ash, half burned, buried or a combination of various conditions. so that it is necessary to use identification methods that have a high degree of accuracy and resistance to various
Identification through DNA analysis has several advantages over other identification methods because DNA testing is more specific, more stable, can be propagated in vitro and distributed throughout the body cells. In the aspect of forensic medicine, DNA profile analysis also plays a role in the identification of the perpetrators of murder, sexual violence, victims of mutilation, victims of mass disasters and determination of offspring.

In population studies, analysis of genetic variations based on DNA profiles plays a role in analyzing human origins, migration, and evolution. It also can be used to find out the genetic structure, variation and affinity of a population. From the point of view of forensic genetics, population is defined as a group of humans from the same common ancestor. When discussing in the context of populations, there are differences in DNA sequences known as polymorphisms that can occur in the part of the DNA that encodes or the part that does not encode the protein. DNA polymorphisms can also refer to very diverse parts of chromosomes from one individual to another.

Genetic variation is a genetic difference between individuals which is inherited from one generation to the next, so that it can be used as a differentiator between one individual and another individual. Conversely, if there are two people whose DNA profiles are similar, it could be that the kinship between the two people is very close, maybe one mother, one grandmother, or one ancestor, therefore DNA polymorphism can be used in making genetic maps of a population. The existence of a genetic map of a population obtained from different DNA profiles can help track the similarities between victims and existing data. The purpose of the analysis of variations genetic (polymorphism) in the case of forensics is to find a DNA profile that has a high level of discrimination, ideally it will produce a unique DNA profile for each individual. This is very useful for matching biological evidence from the scene of a crime to the identity of someone with a high degree of trust and can be a very strong forensic evidence.

In October 1998, the Federal Bureau of Investigation (FBI) began a DNA mapping project using the Combine DNA Index System (CODIS). This index is not a database of criminal records but is a system that provides genetic information that is needed to match medical evidence with suspected victims or perpetrators. The FBI has recommended DNA analysis using the CODIS STR loci in the identification process.

Until now the genetic variation data and specific STR loci for identification of the Banjar Hulu tribe is unknown. By conducting DNA analysis through the STR CODIS locus, it is expected to be able to find and explain the genetic variation of the Banjar Hulu tribe, and the specific STR loci for identification of the tribe. The existence of genetic variation data is expected to help the process of identification of forensics of unknown individuals, especially in the event of a mass disaster that occurred in Kalimantan. The aim of the study are identify and analyze the genetic variation of Banjar Hulu tribe through nucleus DNA examination of short tandem repeat (STR) Loci combined DNA index system (CODIS)

Methods

This type of study is descriptive observational which is one type of study that aims to provide an explanation and real facts in the field that depend on the situation about the genetic variation of the Banjar Hulu Tribe. The design of this study is cross sectional or momentary because each sample is only examined once. The population of this study is the Banjar Hulu Tribe. The sample size is calculated by the Lemeshow formula (1997). From the sample size calculation, the minimum number of samples needed for the Banjar Hulu Tribe is 35 people. The sampling technique with Probability Proportional to Size (PPS) is a sampling technique that highly considers the size of the group or population. The sample of this study came from the Banjar Hulu Tribe who lived in Banua Kepayang Village, Labuan Amas Selatan District, Hulu Sungai Tengah, South Kalimantan Province.

The sample of this study has an inclusion and exclusion criteria.

Inclusion criteria, namely:

1. Men or women who agree to be the subject of study
2. Minimum age 21 years old.
3. Healthy born and inner
4. A native descendant who has ties up to 3 generations above with the Banjar Hulu Tribe.

Exclusion Criteria, namely:
1. Men or women who have a phobia of needles
2. Men or women who have indications of psychiatric disorders

Sampling was carried out in Banua Kepayang Village, Labuan Amas Selatan District, Hulu Sungai Tengah Regency, South Kalimantan Province. The study was conducted at the Laboratory of Genetics of the Institute of Tropical Disease (ITD) Airlangga University Surabaya, March 2018 to September 2018. This study was conducted in two stages, first obtaining information on the family tree from study subjects conducted by interview, then the second stage was carried out by taking blood on study subjects that fit the criteria. Before conducting the study, an ethics feasibility test must first be conducted and declared to have passed an ethics feasibility test.

At the time of the interview, the subjects were given an explanation of the study procedures and filled out informed consent. Then the subjects were interviewed and genealogy drawing was made from the subject. Blood sampling is performed on eligible subjects. Blood sample is taken from peripheral blood from vein mediana cubiti. Then blood sample is analyzed and DNA examination is done.

The selection of DNA extraction method with DNAzol because this method is practical and easy to implement. Measuring the level and purity of DNA through the UV-Spectrophotometer. For PCR DNA amplification TR and GM nuclei used STR CODIS primers (CSF1PO, D18S51, D21S11, D21S11, D3S1358, FGA, D8S1179, D5S818, D7S820, D13S317, D16S539). On DNA PCR results that have been amplified using STR CODIS primers were electrophorized using a polyacrilamide composite gel to determine the success of DNA amplification and continued analysis of each electrophoresis result. Data were analyzed based on the frequency of the allele description of each locus.

Results and Discussion

In the results of this study it is known that the Banjar Hulu Tribe has a variety of alleles at several loci. At the TPOX locus the Banjar Hulu tribe has a dominant allele of allele 8 and 9 of 91.43% and the lowest allele of allele 6 and 7 of 0%. The THO1 locus of the Banjar Hulu tribe has a dominant allele, which is allele 9.3 as much as 100% and the lowest allele is allele 10.3 and allele 11 is as much as 2.86%. The Banjar Hulu tribe has a distribution of all alleles at the THO1 locus, namely alleles 8.3, 9, 9.3, 10, 10.3 and 11. The Banjar Hulu FGA locus found dominant alleles, namely allele 21 as much as 40% and the lowest allele was 18.3, allele 19.3, allele 20.2, allele 21.2, allele 23, allele 23.1, allele 23.3, and allele 25.2 as much as 0%. At the CSF locus of the Banjar Hulu Tribe, it is known that the dominant allele is allele 29.2 as much as 45.7% and the lowest allele is seen in allele 8, allele 10.3, allele 11 and allele 28.2 which is as much as 0%. Locus D5S818 have a dominant allele of the Banjar Hulu tribe, namely allele 11 as much as 77.14%. The lowest allele is in allele 9 as much as 2.86%. The Banjar Hulu tribe has all the alleles at the D5S818 locus as can be seen in the picture. The VWA locus have a dominant allele that is the allele of the Banjar Hulu 16 Tribe as much as 91.43% while the lowest allele is found in alleles 13 and 15.1 which is as much as 0%. Locus D18S51 Banjar Hulu tribe has two dominant alleles namely 15 and 16 alleles as much as 37.14% and the lowest alleles are allele 9, 13, 18 and 20 as much as 0%. The D21S11 locus have a dominant allele in the Banjar tribe that is allele 31 as much as 42.86% and the lowest allele at allele 34 as much as 0%. The D3S1358 locus has a dominant allele that is allele 16 in the Banjar Hulu Tribe by 100% and does not have an allele 15.2 at the D3S1358 locus. Locus D13S317 have a dominant allele that is allele 8 as much as 97.14% in the Banjar Hulu Tribe. The Banjar Hulu tribe has more varied alleles at loci D12S317 allele 8; 8.1; 9; 10; 11; 12. The lowest allele is in allele 11 and allele 12 as much as 2.86%. The D8S1179 locus in the Banjar Hulu Tribe has 100% of alleles 12 and 11.43% of alleles 13. The D16S539 locus of the Banjar Hulu tribe has a dominant allele that is allele 11 as much as 82.86% and the lowest allele is in the 10 allele and 13.1 allele as much as 2.86%. At the D16S539 locus the Banjar Hulu tribe has a greater allele variation of 10; 11; 12; 13; 13.1; 13.3. The D7S870 locus in the Banjar Hulu tribe has a
dominant allele that is allele 9 (34.29%). 7.3 alleles; 8 and 12.1 never appeared in the Banjar Hulu tribe at the D7S870 locus.

Based on the results of study that has been done, the Banjar Hulu tribe has loci and alleles that are owned by everyone in the population, namely locus THO1 allele 9.3, locus D3S1358 allele 16 and locus D8S1179 allele 12. Alleles at these loci are typical markers for the tribe Banjar Hulu. So, to be able to determine an individual is a member of the Banjar Hulu tribal community must refer to the existence of these three loci / alleles.

The Banjar Hulu tribe does not have alleles 6 and alleles 7 at the TPOX locus, alleles 18.3, 19.3, 20.2, 21.2, 23, 23.1, 23.3, 25.2 at the FGA locus, alleles 8, 10, 11 at the CSF locus, alleles 9, 13, 16, 20 at the D18S51 locus, allele 34 at the D21S11 locus, allele 15.2 at the D3S1358 locus, alleles 7.3, 8, 12.1 at the D7S870 locus. Whereas at several loci, the Banjar Hulu tribe has alleles that are normally distributed. At THO1 locus has distribution on all alleles, at allele 8.3, 9, 9.3, 10, 10.3, 11, locus D5S818 at allele 9, 10, 11, 12, 13, 14, 15, 16, locus D13S317 at allele 8, 8.1, 9, 10, 11, 12, locus D8S1179 at allele 12 and allele 13, locus D16S539 at alleles 10, 11, 12, 13, 13.1, 13.3.


For the purposes of forensic identification, the presence of THO1 locus allele 9.3, locus D3S1358 allele 16 and locus D8S1179 allele 12 which are always present in the population of the Banjar Hulu Tribe is very significant. Likewise the TPOX locus at allele 6 and allele 7, FGA locus at alleles 18.3, 19.3, 20.2, 21.2, 23, 23.1, 23.3, 25.2, CSF locus at alleles 8, 10.3, 11, locus D18S51 alleles 9, 13, 18, 20, locus D21S11 allele 34, locus D3S1358 allele 15.2, locus D7S870 allele 7.3, 8, 12.1 which are not found in the population of the Banjar Hulu tribe.

In the results of this study, it is known that the Banjar Hulu tribe has 376 heterozygous alleles and 79 homozygous alleles, this shows that the Banjar Hulu tribe has a heterogamous pattern of marriage. This is in accordance with Daud (1997) which states that the Banjar tribe has an exogamous pattern of marriage.

The Banjar Hulu tribe are the inhabitants who inhabit in the valley area of river branches from the Negara river that flows into the Meratus Mountains. In the beginning their settlement area was a distance from each other, around the settlement there was also a Dayak tribe settlement. Concentration of a large population is located on the banks of the river, at river cliffs that are relatively higher than the surrounding area in the form of swamps. The Banjar Hulu tribe inhabit the Negara, Tabalong, Batang Alai, Labuan Amas, Amandit, Tapin and Martapura areas.

The pattern of exogamic Banjar Hulu marriages is strongly influenced by the teachings of the Islamic religion they profess. Unlawful women are married by men because of hereditary ties consisting of mothers, grandmothers of both fathers and mothers, daughters and their offspring, sisters in line or one father / one mother, daughter, and sister’s daughter. In addition to being related to kinship, there is also a prohibition on marrying brothers and sisters. The Mahram for men are mother-in-law, stepmother, stepmother, daughter-in-law, stepdaughter, and sister-in-law.

Marriage is something that is considered sacred by the Banjar tribe. When a man has reached the time to get married, then the closest family will hold a process of Basusuluh, which is a process to obtain information about the desired prospective wife, which is generally based on the choice of the man with the family’s consent. There are four things that are the object of the Basusuluh namely about her religion, offspring, ability to manage the household and the beauty of her face. About religion, does she come from a descendant who is devout in practicing her religion (Islam). About his descendants, whether she comes from a good offspring, kind, and patient and responsible. About the ability of her household, whether she comes from a rich or underprivileged family, About the beauty of his face,
how is her physical condition. The influence of the teachings of Islam and traditionalism causes Banjarese very rarely married to his closest relatives.26

Geographical and cultural factors, especially marital patterns, as described above can explain the uniqueness and diversity of the genetic identity of the Banjar Hulu tribe and the allele patterns that tend to be more heterozygote.

**Conclusion**

Based on the analysis of the genetic variation of the Banjar Hulu tribe through examination of the nuclei locus of the short tandem repeat (str) combine DNA index system (CODIS), it was concluded that the Banjar Hulu tribe has the characteristics of the presence of THO1 locus at allele 9.3, D3S1358 locus at allele 16 and D8S1179 locus at allele 12, so that the existence of alleles at these loci is a reference to determine the individuals who come from the tribe.

This study has study limitations, so suggestions for further studiers need to do further study with a number of different regions to get genetic variation data from the Banjar Hulu tribe and also need to do study on other tribes to get data on genetic variation of the population inhabiting the island of Borneo.

**Ethical Clearance:** Taken from Medical Faculty of Lambung Mangkurat University Ethical Review Board (No. 615/KEPK-FKUNLAM/EC/III/2018)

**Source of Funding:** Self-Funding

**Conflict of Interest:** None

**Reference**


The Changes in Some Hematological Parameters among University Students Due to Stressful Conditions During and after Examinations Period

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Abstract

Background: Several studies demonstrated that stress can cause an obvious change in the hematological parameters in healthy individuals. In the current study, we determined whether the stress during the exams can produce some hematological changes and how long this effect may take after the end of the exam period.

Methods: Seventy (n= 70) male and sixty (n=60) female student of similar height, weight and age were selected from the department of medical laboratory sciences, Al-Ahliyya Amman university. All students were first had a preliminary medical checkup. Students excluded from the study include those who had a chronic disease and any health issues or habits affecting the studied parameters, such as smoking; fever and high blood pressure. All study participants were between the age group of 20-22 years. Blood samples were taken three weeks before the exam, during and 72 hours after the end of the exam period. Estimations of red blood cells (RBC), white blood cells (WBC), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), neutrophils, lymphocytes and platelets were accomplished.

Results: Compared with pre-examination results the blood samples taken during exams showed a significant decrease in the readings of lymphocytes (p <0.01) , MCV and MCH (p < 0.05). Significant increase in Neutrophil (p < 0.05) and platelets (p< 0.05). Readings taken after 72 hours of the end of exam period showed significant decrease in white blood cell count (p < 0.01) and further significant increase in the platelet count (p <0.001). No significant changes were observed in the readings of Hemoglobin, Red Blood Cell count (RBC), hematocrit and mean corpuscular hemoglobin concentration (p >0.2).

Conclusion: This study concluded that the stress due to examinations is enough to alter certain hematological parameters. The effect of stress on platelets and WBC remained until the end of the exam period. As the increased platelet count may cause health problems for a person, from bleeding problems to the formation of various clots. We do not know for sure how long the increase in platelet and decrease in white blood cells continue as this may cause health problems for people subject to permanent stress.

Kew words: Examination stress, hematological parameters, university students.

Introduction

In any educational system, improving examination performances of students and reducing their failures is the main goal. In order to achieve better outcomes in any educational system, it is necessary to investigate the factors that contribute to the student’s exam performance. Stress is one of the main notable factors that produced by heavy academic load, stress of everyday exams and
absence of relaxation \(^1\). Physiological studies have recommended that stress of any kind will have a significant impact on hematogenic and endocrine systems\(^2\). Stress can also change the blood cell parameters including neutrophils, platelets, eosinophils, monocytes basophils and lymphocytes in healthy individuals as demonstrated in physiological studies \(^3\). Moreover, several studies prove that the immune system is affected by both stress and emotional reactions \(^4,5\). However, complete blood counts, including the hematopoietic system, leukocyte profile or biochemical markers of stress are all mostly used to recognize possible changes in the immunological system \(^6\). Some other published study demonstrated that stress can impact the number and quality of the cells, such as; leukocyte, platelet and erythrocyte populations which need constant replication to maintain their counts, as in the normal situation these cells characterized a constant turnover of cells \(^7,8\). Stress due to university examinations significantly increases the readings of red blood cells and hemoglobin \(^3\).

A published study on the occupational noise that cause a type of stress on the individuals exposed to noise for several years. Exposure to noise can lead to increase stress, tissue dysfunction, and cause changes in the normal process of secretion of the body’s hormones, thus resulting in significant effects on blood parameters and health\(^9\). This study showed that some hematological parameters such as; MCV, MCH, FBS, and MCHC were declined. While other parameters including cholesterol, RBC, WBC, Hb, and Hct were induced compared with the administrative workers. Therefore, it is obvious that there are noteworthy changes on blood parameters of these workers, which can have harmful effects on their health in the future \(^10\). The literature demonstrated a study mainly focused on observing the changes in hematological parameters and serum cortisol levels after examinations conducted in the medical profession on 1\(^{st}\) year medical students. This result, suggesting that stress is responsible for the changes of these parameters. This published study recommended that proper care and support from both the faculty and the parents is provided, as it is essential for the improvement of the immune system and allow the students to adapt sufficiently to exam stress \(^11\). However, another study focused on investigating the effects of noise and chemical stress on hematological and biochemical parameters of the human body through comparing two groups of people involved in maintenance and transport related work and the other group involved in more responsible duty, working under a continuous chemical and noise stress environment. The results clarify a clear influence of chemical and noise stress on the human body in terms of hematology and biochemistry through showing an increase in total serum protein level, serum urea level, and platelet count in stress exposed group beside a significant decrease in serum triglyceride level in the same group. In addition, it was observed that there is statistically insignificant increase in the count of blood Red blood cells (RBC), White blood cells (WBC), Hematocrit, Mean corpuscular hemoglobin concentration (MCHC) and mean cell volume (MCV) in stress exposed group than normal group \(^12\). As this project helps to understand the role of important hematological parameters in monitoring the stressful conditions, which could affect the student behavior during the exam. Better understanding of the quality of life issues could assist the students to manage their stress in order to perform better in their exams. Our study involved one hundred thirty students \(n=130\) students from both sexes. The blood sampling was conducted before, during and after the exam. However, the study of hematological is essential to assess the physiological status in human. For a fully understand the changes in the mentioned parameters, we have to know the baseline physiological parameters in order to compare the results with the students under stress.

Generally, we are expecting that the parameters investigated during the exam will be different from the parameters measured before or after the exam. The current study focused on the undergraduate students in the 3\(^{rd}\) year and our main target is to observe the changes in hematological parameter including Red blood cells (RBC) count, haematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) count and platelets.

**Methodology**

**Study design and subjects**

The present study was conducted at Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, at Al-Ahliyya Amman University/Amman. A random group was chosen from the third
year students in the College of Medical Sciences. All students were first had a preliminary medical checkup. Students excluded from the study include those who had a chronic disease or any health issues or habits affecting the studied parameters, such as smoking; fever and high blood pressure. Finally, seventy (n= 70) male and sixty (n=60) female students of similar height and weight were included in this study between the age group of 20-22 years. All participated students were informed about all the procedures and outcomes of the study.

**Complete blood profiles**

Five milliliters of blood were taken from each student. It was transferred into lavender vacutainer, containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood count was performed on an automated hematology analyzer (Sysmex XP-300) at Sysmex Corporation, Japan. Each count presented the data of white blood cell count (WBC), red blood count (RBC) platelets count (Plt), mean corpuscular volume (MCV), measurement of hemoglobin (Hgb), hematocrit (Hct), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The determination of blood count was performed for each student three weeks before exam period, during exams and 72 hours after the end of the exam period.

**Statistical Analysis**

The data were analyzed and determine the relationship between various parameters, The un-paired t-test was applied to determine statistical significance. Results were presented as means ± SD. Values <0.05 were regarded as statistically significant.

**Results and Discussion**

This study was carried out to investigate the changes in hematological parameters including red blood corpuscles (RBCs), white blood cells (WBCs), haematocrit, haemoglobin and related RBC indices. A total 130 undergraduate students in the third year medical laboratory sciences were participated in this study. All study participants were between the age group of 20-22 years. The results from this study revealed a significant decrease in WBS count (5.5 × 103 /µl ± 1.9, p < 0.01) 72 hrs. after the end of exam period exam compared with that before exam period (8.6 × 103 /µl ± 2.5) as shown in (Table 1). This such decrease in WBC agreed with the studies of Bopda B et al, Qureshi F et al and Johansson G. et al and contradicts with the reports of Roohi N, Irfan S and Venkappa S, Vasudeva M. The lymphocyte counts

<table>
<thead>
<tr>
<th>Hematological parameter</th>
<th>Before exam ±SD</th>
<th>During exam ±SD</th>
<th>72 hrs. after exam end of period ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells × 103 /µl</td>
<td>8.5 ±2.5</td>
<td>8.8± 3.2</td>
<td>5.5 ± 1.9</td>
</tr>
<tr>
<td>Relative lymphocytes count (%)</td>
<td>33.6 ±4.2</td>
<td>26.8 ± 2.2</td>
<td>31.7 ±3.6</td>
</tr>
<tr>
<td>Relative neutrophil (%)</td>
<td>61.3 ±3.8</td>
<td>68.2 ± 4.7</td>
<td>63.3 ±2.7</td>
</tr>
<tr>
<td>Platelets count × 106 µl</td>
<td>275.6 ±42.4</td>
<td>322.1± 35.5</td>
<td>365.8±48.2</td>
</tr>
</tbody>
</table>
(% ) showed significant decrease during the exam period 
(26.8 ±2.2, p <0.01) whereas the neutrophil counts 
(%) increased significantly during the exam period 
(68.2 ± 4.7, p<0.05) compared to that before the exam 
which agreed with the reports of Viktoriya M. et al 18 
and Nakamura H. et al19. The decrease in the absolute 
numbers of lymphocytes cells has been associated 
with chronic stress which results in redistribution of 
lymphocyte cells and caused them to adhere to the 
20 found an increased number in neutrophils in case of 
chronis stress and attributed this to the increase in both 
acute phase reactants and the functions of TNF-alpha. It 
is very interesting to find a significant increase (322.1× 
106 /µl ±35.5, P<0.05) in platelet count (Table 1) during 
the exam and there has been a further high significant 
increase (365.8× 106 /µl ± 48.2, p<0.001) in their count 
72 hours after the end of the exam period. Their counts 
returned to what they were before the exam (Table 1) 
as none of the previous studies ( (Bhaskara K.3 and 
Johansson G. et al.15) recorded this result, but all were 
satisfied with investigating the effect of stress before 
and during the exam period. We believe that one of the 
reasons for the persistence of high platelets after the end 
of the exam period is the continued rise in the adrenaline 
hormone, which increases as a result of stress. Lande K. 
et al21 showed a close relationship between the hormone 
of adrenaline and the increase in platelet count. We 
think that students during exam may suffer from sleep 
deprivation and there is some evidence (Bangasser D. et 
al 22 and Sharma N, Gupta V 23) that sleep deprivation 
and the increased secretion of cortisol, which is linked 
to the extent of stress, both suppress the immune system, 
which in turn explains a decrease in efficiency of the 
immune function causing a decrease in both WBC 
and lymphocyte counts. Also WBC and Lymphocytes 
possess receptors for adrenaline as its plasma levels 
increase in response to stress. Adrenaline reduces the 
flow of white blood cells, causing a redistribution of 
WBC and lymphocytes between the blood and other 
immune compartments ( Dhabhar F et al. 24) causing the 
free circulating WBC and lymphocytes to decrease. The 
present study showed a significant increase in neutrophils 
during exam period that consistent with the results of 
Bopda B. et al13 and Qureshi F. et al14. We think that the 
type of lifestyle, dietary system and family conditions 
may explain the variable change in the level of stress 
in those students, which is reflected in the results of 
hematological parameters. Pre-examination stress could 
be a quite common condition among University students. 
Several studies were carried out and the changes that 
occur as a result of stress have not been identified fully.

Table 2. Change in Hemoglobin, Red blood cell count, Mean corpuscular volume MCV, Hematocrit percentage, Mean corpuscular hemoglobin MCH and Mean corpuscular hemoglobin concentration MCHC were determined before the exam, during exam and 72 hrs. after the end of exam period. Results show the mean value of 130 students. The significant difference between the count before, during and 72 hrs. after exam is indicated as not significant (NS), *p< 0.05.

<table>
<thead>
<tr>
<th>Hematological parameter</th>
<th>Before exam ±SD</th>
<th>During exam ±SD</th>
<th>72 hrs. after exam end of period ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.4 ±2.2</td>
<td>14.7 ±2.3</td>
<td>14.4 ±1.9</td>
</tr>
<tr>
<td>Total red blood cell count × 106 µl</td>
<td>5.1 ±3.2</td>
<td>5.6 ±3.4</td>
<td>5.2 ±2.8</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin MCH (pg)</td>
<td>27.3 ±2.2</td>
<td>24.4± 2.3</td>
<td>27.7 ±2.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.3 ±3.7</td>
<td>43.7 ±3.2</td>
<td>43.1 ±2.9</td>
</tr>
<tr>
<td>Mean corpuscular volume MCV (fl)</td>
<td>82.5 ±2.4</td>
<td>74.3±3.8</td>
<td>77.5 ±5.3</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration MCHC (g/dl)</td>
<td>35.1 ±0.81</td>
<td>35.3 ±0.98</td>
<td>35.4 ±1.1</td>
</tr>
</tbody>
</table>
The present study demonstrated a significant decrease in the level MCH and MCV during the exam period (24.4 ± 2.3, p<0.05, 74.3 ± 3.8. P< 0.05) respectively which is consistent with the report of Eun et al., this such decrease was explained by Hisham W and Mohammed A. This deficiency in Glutathione reductase which plays an important role in protecting biological cell membranes of RBC against oxidative damage that results from stressful conditions. Their counts returned to what they were before the exam period (Table 2), which undoubtedly confirm the effect of stress on these parameters. There was no change in the levels of Hb, MCHC and hematocrit all through the study (Table 2) which contradicts the studies carried out by Bopda B et al. and Qureshi F. et al. that recorded a decrease in the levels of these parameters during the examination session.

In this study it was observed that stress affects significantly more female students (75%) than a male one (53.3%). This corresponds to previous report of Lande K. et al. on the impact of stress on women more than men as the emotional nature of a woman plays a role in a woman’s sense of stress and anxiety more than men.

**Conclusion**

A large number of students of the university were affected by examination Stress especially during the exam. The study sample participated in this study showed stress that affects some of the hematological parameters. Our study investigated the effect of stress after the end of the exam period, which none of the previous studies addressed that. This study showed that platelets increased and WBC decreased after the end of the exam period and we do not how long they can take to return to normal level. This result about platelets may explain the fact that the stressful people as exposing themselves to continuous rise in platelets and the risk of high blood clot.

**Author Contributions:** Jehad .Al-Homoud and Husni Farah conceptualized and supervised the article. Jehad .Al-Homoud wrote the initial draft and revised subsequent drafts. Talal .Al-Qaisi ,Ali Atoom , ghaleb Oriquat and Khalid Al-Qaisi analyzed and edited the manuscript prior to submission. Wala,a Hamdan did the practical work in the lab. All authors have read and agreed to the published version of the article.

**Funding:** This research received no external funding.

**Ethical statement:** Based on the Ethics Committee’s decision of Al Ahliya Amman University/Jordan dated 08/10/2019, reference number 1/1/2019/2020 (578) it was agreed to carry out the experiments necessary to complete this research

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


Nurse Educator-Led an Educational Intervention on Critical Care Nurses’ Knowledge Regarding Endotracheal Suctioning

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Abstract

Background: Studies placed a great emphasis on implementing in-service educational intervention for nurses in order to improve their knowledge regarding critical nursing procedures. Therefore, critical-care nurses need to be more knowledgeable and competent in providing patient care. Hence, the researcher (nurse educator) developed structured educational program that mainly focused on endotracheal suctioning based on evidence-based recommended practice guidelines. Endotracheal suctioning is vitally important nursing procedure in terms of maintaining airway patency and preventing complications. Objectives: to evaluate the effectiveness of endotracheal suctioning educational program on critical-care nurses’ knowledge. Methodology: a quantitative, quasi-experimental one group pre-test and post-test design was used. Convenience sample of 87 critical-care nurses are recruited from three hospitals at AL-Najaf AL-Ashraf City. The study conducted over the period of time that started on December 10, 2019 and ended on January 22, 2020. Results: most of the participants had inadequate to moderate knowledge at pre-test. While after implementing the educational intervention, the participants gained more knowledge and statistical findings indicated that all nurses have adequate knowledge regarding endotracheal suctioning. Conclusion: Endotracheal suctioning is a widely performed technique for mechanically ventilated patients in critical care units. Nurses must be competent and well educated to perform this procedure in order to prevent patient potential complications and achieve best outcomes. Educational intervention that led by nurse educator has a positive influence on nurses’ knowledge. Recommendations: the researchers are highly recommended implementing ongoing educational program by using recent clinically recommended evidence that focuses on areas where knowledge expansion can be achieved to staff nurse. In Iraq, creating postgraduate degree focusing on nursing education is a cornerstone to engage in the health care team and articulate new scientific approach by using researches in the clinical setting and apply research findings in clinical practice.

Keywords: Critical Care Nurses; Effectiveness of Educational Program; Endotracheal Suctioning; Knowledge; Nursing Education.

Introduction

Patients who admit to the hospital are suffering from a wide range of health problems. Some patients can be critically ill and require immediate interventions to save their lives and prevent deteriorations. It is, consequently, imperative that well educated and well trained nurses provide high quality of nursing care for critically ill patients to overcome complications of complex health status. Critically ill patients are commonly hospitalized in the intensive care units (ICUs). These patients are usually exposed to adverse effects as a result of therapeutic or diagnostic procedures. The role of nurse is not only providing comprehensive high quality critical care, but also preventing complications and working effectively on restoring patient to steady state of health condition.

However, one of the most performed procedures in critical care units is endotracheal suctioning (ETS). Suctioning is a crucial nursing procedure that maintains a patent airway by aspiration accumulated secretions from respiratory system to keep its function and effective gas exchanges. Many researchers have asserted that there are
discrepancies and variations among critical-care nurses’ knowledge and practices regarding ETS. Therefore, inadequate knowledge and lack of commitment related to ETS of recent evidence-based recommended practice guidelines are existed and may jeopardize patient safety. Mwakanyanga et al., (2018) emphasized on the need of continuing education program for ICU nurses in order to enhance their knowledge and bridge the gap between practice guidelines and actual practices. Another clinical trial conducted by Gilder, Parke, and Jull, (2018) has also demonstrated that educational intervention regarding ETS is crucial to foster nurses’ knowledge and achieve desired patient outcomes.

Need of the study:

Unluckily most of ICU patients suffer from complications associated with ETS. This can increase burden on both patient and hospital. In order to prevent or minimize ETS related complications, structured educational program has been designated to improve nurses’ knowledge in order to provide quality care. In spite of many studies implemented educational program, there is no single study in Iraq that provided educational intervention to critical care nurses in regard to ETS. This study is intended to evaluate the effectiveness of educational program on critical care nurses’ knowledge regarding ETS of patients who are mechanically ventilated in hospitals at AL-Najaf AL-Ashraf City.

The research question posed for the current study is “Do critical care nurses gain more knowledge after implementing educational program regarding ETS as compared to their pre-test knowledge?” Moreover, we hypothesized that there will be a statistically significant improvement on the level of critical-care nurses’ knowledge regarding ETS after implementing educational program as compared to their level of pre-test knowledge. General System Theory has been adopted as conceptual framework to guide this study.

Materials and Methods

Research Design:

This study is conducted by utilizing a quantitative, quasi-experimental one group of pre-test and post-test design. The quasi-experimental design resembles experimental design but it lacks the true random assignment of subjects into group. In this study also the ETS educational program was the intervention (manipulation) which is the independent variable (IV), and critical-care nurses’ knowledge about ETS is a dependent variable (DV). The study conducted by evaluating nurses’ knowledge at pre-test and on month after implementing ETS educational program (post-test). The study design is formulated as follow:

Pre-test (O1) ® Educational Intervention (X) ® Post-test (O2).

Study Setting and Sample:

The population for this current study is nurses who are working in the selected hospitals. The target population is nurses who are working in critical care units in hospitals at AL-Najaf AL-Ashraf City, Iraq. The accessible population is nurses who are met inclusion and exclusion criteria and who are working in AL-Sadder Medical City, AL-Hakeem General Hospital, and Middle Euphrates Hospital. The sampling technique was a non-probability, convenience sample of 87 out of 172 critical-care nurses who were willing to participate in this study.

The inclusion criteria for sample selection include:

1. All male and female nurses who were working in critical care units at selected hospitals.
2. Critical-care nurses who were willing to participate in this study.
3. Critical-care nurses who were available at the period of data collection.

The exclusion criteria were as follow:

1. Nurses who were holding administrative position.
2. Nurses who were not available during the period of data collection.
3. Nurses who were not working in the critical care units.
4. Nurses who were not attending the ETS educational program.
5. Critical-care nurses who were selected for Pilot
Study

Structuring the Educational Program and Developing Knowledge Tool:

The purpose of developing an endotracheal suctioning educational program is to provide critical-care nurses with a cohesive body of knowledge based on recent evidence-based practice guidelines that is necessary for nursing profession. Extensive literature review has been done to include the most reliable evidences regarding ETS between publications years from 2010 to 2020. The educational program consists three sections: section one briefly describes the anatomy and physiology of respiratory system, section two explains mechanical ventilation (MV) and the most commonly used modes, and section three discusses ETS procedure in detail such as practices prior to suctioning event, infection control practices, practices during suctioning episode and post suctioning practices. The data collection tool has been developed based on each section with main focuses on section three. The tool includes 40 multiple choice questions with 4 options and one correct answer. Consequently, four questions are about anatomy and physiology of respiratory system, six questions are regarding MV, and the main section contains 30 questions about ETS.

The instrument has been developed and exposed to ten experts in nursing and medicine as well as linguistics due to it is written in Arabic format to accommodate all nurses with different level of education qualifications in order to ensure its validity and consistency. The tool includes the most commonly and mandatory performed steps regarding suctioning procedure for patients who are mechanically ventilated and highlights into ETS educational program in terms of concentrating on need to know rather than nice to know.

A pilot study is conducted for the reliability of the instrument and is analyzed by using test retest method, which measured the coefficient of internal consistency. The obtained value for the instrument score was $r = 0.88$.

Method of Data Collection/Procedure:

The approval was obtained from the institutional review board (Scientific Postgraduate Committee University of Babylon/ Faculty of Nursing Council) after completing the approval forms. The formal ethical application forms contained restricted rules to ensure safety and confidentiality for the participants. Moreover, the study approval from the AL-Najaf Health Directorate was also obtained before the actual data collection period. A brief explanation and discussion about the purpose and procedure of the study were given to the participants. Participants are asked to fill the demographic sheet and knowledge questionnaire before implementing ETS educational program. Then, the researchers provide ETS educational program with aid of learning-teaching strategies: lecture, PowerPoint presentation, guided group discussion, handouts, and showing video. Data are collected during December 10, 2019 and ended on January 22, 2020. Nurses’ knowledge is evaluated according to the sum of scores for 40 items (100%) as follow: $0 – 13 (<33\%) = \text{Inadequate knowledge}$, $14 – 26 (36 – 65\%) = \text{Moderate knowledge}$, $27 – 40 (68 – 100) = \text{Adequate knowledge}$. Evaluation of nurses’ knowledge according to the mean of scores: Adequate knowledge (mean 0.68-2.1), Moderate knowledge (mean 0.34-0.67), Inadequate knowledge (mean 0-0.33).

Results

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Rating &amp; Intervals</th>
<th>$n$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 – 24</td>
<td>10 (11.5)</td>
</tr>
<tr>
<td>Mean 31.77</td>
<td>25 – 29</td>
<td>33 (37.9)</td>
</tr>
<tr>
<td>Standard Deviation 7.30</td>
<td>30 – 34</td>
<td>21 (24.1)</td>
</tr>
<tr>
<td></td>
<td>35 – 39</td>
<td>9 (10.3)</td>
</tr>
<tr>
<td></td>
<td>40 +</td>
<td>14 (16.1)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>64 (73.6)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>23 (26.4)</td>
</tr>
</tbody>
</table>
The finding in table (1) shows the average age was 31.77. Moreover, 64 nurses were male (73.6%). Majority of them was holding diploma in nursing (70.1%). Only two nurses out of 87 had attended training/education program. All of participants (100%) stated that there were no available guidelines to follow. Only eight nurses reported they are self-educated and using internet and online journal to update their knowledge. According to the participants’ total work experience, figure 1 illustrated that the majority (33.3%) had less than one year experience in critical care units.

Figure 1: Distribution of Participants’ Work Experience (N= 87).
Table 2: Evaluation of Nurses’ Knowledge at the Pre-test and Post-test (Sub-Domain Based Analysis).

<table>
<thead>
<tr>
<th>Main Studied Domains</th>
<th>Level of Knowledge</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Mean</td>
<td>Eval.</td>
</tr>
<tr>
<td>Anatomy &amp; Physiology of Respiratory System</td>
<td>Adequate</td>
<td>62 (71.3)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>12 (13.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>13 (14.9)</td>
<td></td>
</tr>
<tr>
<td>Mechanical Ventilation (MV)</td>
<td>Adequate</td>
<td>13 (14.9)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>31 (35.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>43 (49.4)</td>
<td></td>
</tr>
<tr>
<td>Endotracheal Suctioning (ETS)</td>
<td>Adequate</td>
<td>7 (8.0)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>40 (46.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>40 (46.0)</td>
<td></td>
</tr>
</tbody>
</table>

Eval. = Evaluation; Adequate knowledge = (0.68-2.1), Moderate knowledge = (0.34-0.67), Inadequate knowledge = (0-0.33).

Table 2: The knowledge of critical-care nurses is evaluated and statistically analyzed in three domains: anatomy and physiology of respiratory system, MV, and ETS as demonstrated in table 2. The statistical finding indicates that there is an improvement in nurses’ knowledge after applying ETS educational program in all aspects.

Table 3: Overall Evaluation of Nurses’ Knowledge at the Pre-Test and Post-Test.

<table>
<thead>
<tr>
<th>Periods of measurements</th>
<th>Level of Knowledge</th>
<th>n (%)</th>
<th>Overall Mean</th>
<th>Overall Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>Adequate</td>
<td>13 (14.9)</td>
<td>0.53</td>
<td>Moderate Knowledge</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>48 (55.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>26 (29.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (N)</td>
<td>87 (100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-test</td>
<td>Adequate</td>
<td>81 (93.1)</td>
<td>0.84</td>
<td>Adequate Knowledge</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>5 (5.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>1 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (N)</td>
<td>87 (100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The above table (3) shows that overall level of participants’ knowledge before and after implementing ETS educational program. The finding demonstrated that the participants’ knowledge enhanced significantly after giving ETS educational program with the overall mean score (0.80). This is evident that the educational intervention was effective.

**Table 4: Subdomain and Overall Mean Difference of Critical-Care Nurses’ Knowledge (Paired T-Test) throughout Two Periods of Measurements (Pre-test and Post-test).**

<table>
<thead>
<tr>
<th>Main studied Domains</th>
<th>Period of Measurements</th>
<th>Mean</th>
<th>SD</th>
<th>t-value</th>
<th>d.f</th>
<th>p-value</th>
<th>IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomy and physiology</td>
<td>Pre-test</td>
<td>.712</td>
<td>.293</td>
<td>5.354</td>
<td>86</td>
<td>0.0001</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>.907</td>
<td>.180</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>Pre-test</td>
<td>.480</td>
<td>.250</td>
<td>10.525</td>
<td>86</td>
<td>0.0001</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>.806</td>
<td>.199</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endotracheal suctioning</td>
<td>Pre-test</td>
<td>.397</td>
<td>.200</td>
<td>18.195</td>
<td>86</td>
<td>0.0001</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>.836</td>
<td>.115</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Evaluation</td>
<td>Pre-test</td>
<td>0.53</td>
<td>0.18</td>
<td>15.861</td>
<td>86</td>
<td>0.0001</td>
<td>31%</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>0.84</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD = Standard Deviation; HS = Highly Significance; IR = Improvement Rate = ($Mean$ at the posttest - $Mean$ at the pretest)*100.

**Discussion of the Results**

According to gender the majority of participants (73.6%) were male and the remaining (26.4%) were female. This finding supports Majeed (2017) who conducted a research study to assess intensive care unit nurses’ knowledge and practices regarding ETS and reported that the majority of studied nurses were male (70%). According to subjects’ educational qualification majority (70.1%) of the respondents were holding diplomas in nursing. This agreement with Aboalizm and Elhy (2019) who found that diploma nurses are more common than any other degree nurses in critical care units.4

Furthermore, findings from this study demonstrated that the majority of the sample (97.7) did not participate previously in training or educational program regarding ET suctioning and only (2.3%) have attended educational program. This also is indicated in Haza’A, Mohammed, Abdel-Aziz, & Ibrahim (2015) study who stated that the majority of ICU nurses (73.3%) have not attended any previous instructional or training program regarding ETS.5 Another study by Varghese & Moly (2016) also reported that the majority (78%) of critical-care nurses did not attend training program about ETS previously.6
The findings from this study indicated that all participants did not follow ETS guidelines (100%). Several researchers identified that in spite of many published practice guidelines on suctioning but nurses are unaware of current evidence-based practice recommendations and clinical performance is frequently based on experience, habitual and tradition as contrasting to empirical evidence. Gilder, Parke and Jull (2018) stated that accessible practice guidelines on suctioning intubated patients are not being applied into most clinical settings worldwide. Availability of most recent evidence-based practice guidelines regarding endotracheal suctioning procedure may not be enough to ensure the accurate implementation by nurses, there must be training or education program focusing on step by step performing the procedure as well as emphasizing on the benefits of correct application to prevent avoidable hazardous complications.

According to participants’ statements, almost all of critical-care nurses (90.8%) did not take a self-education responsibility regarding their fundamental tasks as a critical-care nurse, only (9.2%) of them utilized internet and research journals to update their knowledge based on evidence-based current practice. This could be one of the main factors of having poor to moderate knowledge. Regarding the critical-care nurses’ work experience, the result showed that (33.3%) had less than one year experience in critical care units.

Concerning to critical-care nurses’ knowledge before implementation of ETS educational program, the current study findings show that overall knowledge score at the pre-test was moderate. The overall mean pre-test knowledge score was (0.53) as indicated in table (3). This finding supports the study conducted by Ansari, Alavi, Adib-Hajbagheri, and Reza, (2012) that aimed to assess nurses’ knowledge and practices regarding endotracheal suctioning in ICUs of Kashan Hospital. The researchers found that (47.8%) of nurses had moderate knowledge. Another study that was conducted by Parihar (2015) has also shown that pre-test nurses’ knowledge in field of endotracheal suctioning was average with the mean score (17.07). Moreover, several research studies had also shown that most of nurses had poor to moderate knowledge regarding suctioning procedure before delivering educational program. The reason for that could be because almost all the participants of this current study did not receive ETS educational program previously.

Notwithstanding, the most interesting result was found after implementation ETS educational program. There was a remarkable enhancement of knowledge among studied percipients after providing educational intervention. The result showed that overall knowledge score after educational intervention was adequate. The overall mean post-test knowledge score was (0.84) as illustrated in table (3). The findings of post-test confirmed the effectiveness of ETS educational program in terms of significantly improving critical-care nurses’ knowledge. This result corresponds well with studies carried by Aboalizm & Elhy (2019); Sharma et al, (2014); and Vinayaka and Bernet (2016), who noted that following educational or teaching program significant improvement were perceived in knowledge level as compared to preliminary assessment.

The continuing nursing education department in each hospital should place a great emphasis on providing in-service education program periodically in order to embrace change, bear accountability, and enhance nurses’ knowledge in terms of achieving desirable outcome. The education and training strategies are two essential components of staff development that occur after nurses’ indoctrination.

Regarding to subdomain analysis of knowledge, the results showed that critical-care nurses had an adequate knowledge at pre-test regarding anatomy and physiology of respiratory system with the overall mean score was (0.71). Whereas after presenting ETS educational program (post-test), the level of nurses’ knowledge raised to higher score with the overall mean was (0.90) as shown in table (2). This enhancement in nurses’ knowledge in field of anatomy and physiology of respiratory system was due to an appropriate focusing of the educational intervention that led by the researcher. Additionally, this supports the findings of a previous research study conducted by Elsaman (2017) who found that improvement of participants’ knowledge after providing endotracheal suctioning guidelines which reflected on patients’ respiratory status. Another study in Egypt that was carried by Hassan et al, (2018) aimed to determine the effect of educational program
on nurses’ knowledge regarding endotracheal tube of adult patients. The researchers declared that there were highly statistical significant differences between pre and post implementation of educational program regarding anatomy and physiology of respiratory system with the P<0.0001. This confirms our finding in terms of enhancing nurses’ knowledge.

Concerning the knowledge of mechanical ventilation (MV) aspect, the results of this study showed that most of the respondents (n=43, 49.4%) had inadequate knowledge at pre-test. While at the post-test, the majority of the nurses (n= 54, 62.1%) had an adequate knowledge as showed in table (2). Similarly, the study conducted in Australia by Guilhermino, Inder, Sundin, and Kuzmiuk (2014) which aimed to determine the effect of educational resources provided regarding invasive mechanical ventilation on novice and expert ICU nurses’ knowledge. The researchers underlined that the vast majority of ICU nurses (63%) did not receive education about MV before recruiting in ICU and their knowledge was limited, but after providing education regarding MV the participants’ perceived it as valuable and beneficial for safe and effective practices.

Thus educational intervention plays a pivotal role in improving knowledge and practicing safe care to critically ill patients who are under MV. Knowing the working principles and settings of MV can prevent avoidable complications that may happen during suctioning episode. Critical-care nurses must be aware that inappropriate mode of MV may jeopardize patients’ health status. Intubated mechanically ventilated patient requires continuous monitoring to keep patent airway and intervene rapidly when needed. Hence, providing periodic continuing education strategy regarding MV is an essential aspect of promoting nurses’ competencies.

Regarding nurses’ knowledge about endotracheal suctioning (ETS), the results of current study showed that 40 nurses (46.0%) out of total sample 87 (100.0%) had an inadequate knowledge, 40 nurses (46.0%) had moderate knowledge and only 7 (8.0%) had an adequate knowledge before implementing ETS educational program at pre-test period. Consequently, the total mean score of nurses’ knowledge in field of ETS was (0.40). Meanwhile, after implementing the structured ETS educational program at post-test period, the statistical result of nurses’ knowledge regarding endotracheal suctioning aspect showed that the vast majority (n = 81, 93.1%) of participants had an adequate knowledge, five nurses (5.7%) had a moderate knowledge and only one nurse (1.1%) had an inadequate knowledge. Therefore, the overall mean score of nurses’ knowledge concerning ETS at post-test was (84), and these results are portrayed in table (2).

The statistical significance found congruent with the study carried by Harjot, Kumar and Krishan, (2016) that aimed to assess the effectiveness of teaching intervention on nurses’ knowledge and practices regarding ETS. The researchers concluded that teaching intervention implied positive change on nurses’ knowledge and practices. The authors reported that there was a statically significant score between nurses’ knowledge before and after application the teaching program regarding ETS. The mean score of pre-test was (19.23±4.180), while the post-test mean score was (27.26±4.046). This is evidence that educational intervention has the potential to increase knowledge of staff nurses regarding ETS procedure.

**Conclusion and Recommendation**

Our ETS structured educational program proved its effectiveness by articulating highly statistical significant score after implementation in terms of increasing level scores of critical-care nurses’ knowledge. The main focusing of our educational intervention was to examine its effectiveness in terms of improving nurses’ knowledge regarding suctioning procedure. We hypothesized that there will be a statistically significant improvement on the level of critical-care nurses’ knowledge regarding ETS after implementing educational program as compared to their level of pre-test knowledge. Accordingly, the research hypothesis is accepted at (P = 0.0001) level of highly significant and null hypothesis is rejected as illustrated in table (4). According to participants’ knowledge scores after implementing ETS educational program, our finding indicated that there was a statistically significant improvement as compared to baseline variables (pre-test) table (4).

Therefore, there are two conceivable explanations for this result: The first explanation according to Bertalanffy’s general system theory (GST), to improve individual (nurse) knowledge and practices the emphasis
should be designed on information (energy) in order to assist nurses in terms of knowledge acquisition. The selection of GST was best choice to guide this study due to it draws an avenue to guide the process of educational intervention. All concepts of the theory were consistent to delineate the research process and its objectives. However, according to the GST the input is conceived as information or material entering the system. The area of input was the ETS educational program that has been designed to meet critical-care nurses’ need in terms of increasing their level of knowledge about suctioning procedure.

The finding indicates that no matter of the level of participants’ educational qualification the objectives are the same and have been met; however, the teaching-learning strategies for the provided educational program were formulated to meet each participant’s learning needs. The educational intervention promoted knowledge integration and critical problem-solving approach which are important in critical care units. The processes within the system that transform the input into output are called as throughput. In our modified conceptual framework, the throughput consists of the desired change in knowledge regarding ETS and was achieved after applying the educational program, which in that situation transformed the input to output. The output is the end product of the system. It consists of information or materials transformed to achieve the system’s goals. In this case, the output reflected the improvement in participants’ knowledge which considered as a part of feedback loop that involves into input again. The feedback concept is the perceiving of attaining or not attaining the preset system objectives. Thus, feedback is crucial in embracing change and is a source of motivation to continue or terminate the activity. In this study, the feedback is considered as a summative evaluation process (post-test) which indicated by statistical results that the ETS educational program was effective in terms of achieving the study’ objectives and answer the research question.

The second explanation could be that the nurse educator (researcher) utilized open-ended questions to allow the nurses to discuss and answer their questions during teaching period with appropriate guidance from the researcher. In nursing education using questions is an important approach to promote critical thinking skills. Furthermore, the cornerstone of nursing profession is nursing diagnosis and the application of nursing process. Hence, the ETS educational program placed a great emphasis on indications, complications, monitoring and application evidence-based recommended practice related to ETS guidelines that play an important role in achieving best outcomes.

We are strongly recommended providing evidence-based practice guidelines and educational sessions that led by competent nurse educator to embrace positive change on best quality care through improvement of nurses’ knowledge.

Strengths and Limitations of the Study:

The strengths of the present research study are adding a body of consolidated knowledge to the previous published research studies that supported the benefit of using ETS educational program in critical care units. The implemented program can be feasible in different healthcare settings and can be provided as a self-learning package. The results of the present study provided the trustworthy and valuable evidence toward applying in-service educational program that enhance nurses’ knowledge. Using recent evidence-based recommended practice guidelines in structuring the ETS educational program was the most important aspect in terms of keeping with up to date of knowledge worldwide. Nonetheless, despite of many research studies regarding the effectiveness of ETS educational program have been conducted internationally and regionally, our study is considered the first study in Iraq that implemented an educational intervention about ETS. Finally, the educational program demonstrated its effectiveness and feasibility in terms of bringing improvement to the suctioning technique and has a positive influence on nurses’ knowledge.

The main limitations of the current study include the following: some of the critical-care nurses were excluded from this study because they were not able to attend the ETS educational program. This study was conducted in three different hospitals in one city. It is suggested that future research include more hospitals in different cities such as Middle Euphrates Governorates hospitals. The small number of critical-care nurses (87) participated in this study limit the generalizability of the finding to other population. It is recommended for future studies
to include a large number of samples. Lastly, most of the participants were holding diploma degrees in nursing. It is recommended that future research include academic and postgraduate nurses.

**Ethical Clearance:** Taken from University of Babylon/College of Nursing Research Ethics Committee, Iraq.

**Source of Funding:** The authors have no sources of funding to declare.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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7. Parihar RR. Effectiveness of planned teaching programme on the knowledge of endotracheal suctioning among staff nurses working in ICU. Baba Farid University Nursing Journal 2015; 8(1), 63-67.
11. Vinayaka AM, Bernet S. A Study to Assess the Effectiveness of Structured Teaching Programme on Knowledge and Practice Regarding ET Tube Suctioning among Pediatric ICU Staff Nurses in Selected Hospital at Bangalore. International Journal of Nursing Education 2016; 8(2), 122.
Relationship of Low Maternal Vitamin D3 Level and Adverse Early Neonatal Outcomes

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Abstract

Background: Low serum vitamin D [25 (OH) D] levels have been shown to have multiple health-related implications in females at childbearing age, during pregnancy, as well as in their children. Aim of the study: To assess the inverse relationship between vitamin D3 level and adverse neonatal outcomes using parameters such as fetal birth weight, head circumference, Apgar scores, fetal respiratory distress syndrome, and rate of neonatal admission.

Materials and methods: A prospective study was conducted on 100 early- and full-term pregnant women at Al-Elwiya Maternity Teaching Hospital in Baghdad from 1st of April 2017 to 31st of March 2018. Maternal vitamin D deficiency was confirmed based on levels <20 ng/mL.

Results: The vitamin D3 levels in the studied pregnant women were categorized as follows: normal (45%), insufficiency (31%) and deficiency (24%). The significant adverse neonatal outcomes associated with vitamin D3 deficiency were low Apgar score (54.2%), low birth weight (91.7%), small head circumference (91.7%), respiratory distress syndrome (66.7%), and neonatal intensive care unit admission (66.7%).

Conclusions: vitamin D3 deficiency among pregnant women is associated with prominent adverse neonatal outcomes.

Keywords: Vitamin D3, term pregnancy, neonatal outcome, Apgar score

Introduction

Hypovitaminosis D is prevalent worldwide, especially in Asia and the Middle East despite their tropical climate with abundance of sunlight. This suggests the prevalence of specific risk factors for hypovitaminosis D in these regions. These include the classic predictors and the conservative concealed clothing style in women in general and in men from Gulf countries in particular. The lack of governmental regulation regarding food fortification with vitamin D in these regions is also a potential risk factor.¹

Literature from Saudi Arabia, Kuwait, United Arab Emirates, and Iran revealed that 10-60% of mothers and 40-80% of their neonates had undetectable to low vitamin D levels (0-25 nmol/L) at the time of delivery.² In Iraq, hypovitaminosis D occurred in more than 65% women of childbearing age.³

A developing fetus is entirely dependent on the mother as a source of vitamin D. About 60 to 70% of the maternal plasma level of 25- hydroxyl-vitamin D [25(OH) D] acts as a source of vitamin D in the developing fetus. However, pregnant women have been reported with an unacceptably high prevalence of vitamin D deficiency and insufficiency.⁴ ⁵ This, in turn, has been reported to increase the risk of adverse maternal and neonatal outcomes.⁶

Many observational studies have indicated that maternal hypovitaminosis D (as defined by maternal
25(OH) D levels <20 ng/ml) is a significant risk factor for adverse neonatal outcomes including underdevelopment according to gestational age, preterm birth, detrimental effect on bone and teeth development, etc. 4, 7 Several studies have associated low 25(OH) D level to the risk of respiratory and other infectious diseases. 8 Vitamin D deficiency also puts children at a higher risk of diseases such as asthma and sepsis. 9, 10

Many Iraqi researchers have reported significant adverse effects of hypovitaminosis D during pregnancy like preterm labor, preeclampsia and risk of gestational diabetes mellitus. 11, 12 In addition, optimum vitamin D level has been shown to have a vital role in limiting the occurrence of preeclampsia and other hypertensive disorders during pregnancy. 13

Aim of Study

To assess the inverse relationship between vitamin D3 levels and adverse neonatal outcomes, especially regarding fetal birth weight, head circumference, Apgar scores, fetal respiratory distress syndrome, and neonatal admission.

Patients and method

Design, settings and sampling

This prospective clinical study was conducted at the Al-Eleiya Maternity Teaching hospital, Baghdad, Iraq between first of April 2017 and 1st of March 2018.

Ethical consideration: Oral informed consent and signed paper was taken from each participant prior to their enrollment in the study.

All pregnant women admitted to the obstetrics wards at Al-Elwiya Maternity Teaching Hospital for elective cesarean section were enrolled in the study. A total of 100 term pregnant women were selected based on the inclusion and exclusion criteria.

The inclusion criteria included women with singleton and full-term healthy pregnancies.

Women presenting with preterm, multiple pregnancies, congenital abnormalities, taking any long-term drugs, smoking habit, obstetrical or medical complications (diabetes, pre-eclampsia, anemia, antepartum hemorrhage, premature rupture of membrane, polyhydramnios, etc.) were excluded.

Cesarean section of all participants was carried out under spinal anesthesia to eliminate any side effects of general anesthesia on the neonate. All participants were asked questions according to a special questionnaire prepared specifically for the study based on previous similar studies.

A detailed history was obtained from women, including the age, occupation, residence, and clothing style of selected pregnant women.

A detailed obstetrical history was taken from the participants: It included number of parities and antenatal care visits. Gestational age was determined according to last menstrual period and early ultrasound report; it was confirmed by late ultrasound (which was done to ensure viability and first-time pregnancy and exclude any congenital abnormalities). A thorough physical examination was performed for all the participants to exclude any other underlying pathology.

A 5 ml blood sample was drawn from each participant and was sent for cholecalciferol (25(OH) D3) level analysis to the Laboratory of Al-Elwiya Maternity Teaching Hospital. High-performance liquid chromatography methods quantitated 25-hydroxy vitamin D2 and D3 levels. Confirmation of vitamin D deficiency diagnosis was based on levels <20 ng/mL; vitamin D insufficiency was defined as levels ranging from 20 to 29.9 ng/mL, while normal level was 30 ng/mL and more.

Each neonate was assessed and followed up by a senior pediatrician post delivery or after admission to the neonatal intensive care unit (NICU). All respondents were assessed for weight, head circumference, Apgar score at 1 and 5 minutes, respiratory distress syndrome, and admission to NICU. The Apgar score was classified according to WHO definition into <7 and >7. Neonates with Apgar scores of <7 had compromised vital functions (appearance, pulse, grimace, activity and respiration).

Neonatal weight less than 2.5 kg was regarded as low and was measured using the UNICEF weighing scale.

The head circumference of neonates was classified into small (<34.5 cm for males and <33.8 cm for
females) and normal values (>34.5 cm for males and >33.8 cm for females). Respiratory distress syndrome, NICU admission, and duration of NICU stay were also assessed.

**Statistical Analysis**

MS-Excel and Statistical Package for Social Sciences (SPSS) version 23 were used to collect and analyze the data. Chi-square test was used for comparison between categorical data (Fisher’s exact test was applied when expected variable was less than 20% of total). One-way ANOVA analysis was used to compare more than two means. The level of significance (p value) was set as ≤ 0.05.

**Results**

Vitamin D3 levels of the participants were as follow; normal (45%), insufficiency (31%) and deficiency (24%). *(Figure 1)*

![Figure 1: Vitamin D3 level distribution.](image)

A highly significant association was observed between the age of the pregnant women and vitamin D3 insufficiency (p<0.001), as 96.8% of women with vitamin D3 insufficiency were in the younger age group. In addition, we found that 95.8% of women with vitamin D deficiency were housewives.

It was shown that women with abnormal BMI (overweight and obese) suffered from vitamin D3 deficiency (p<0.001). Pregnant women living in rural areas were significantly associated with vitamin D3 deficiency (p<0.001), (95.8%). A highly significant association was also observed between vitamin D3 deficiency and veiled pregnant women, high parity and irregular antenatal care visits (p<0.001) *(Table 1)*.
Table 1: Distribution of women’s characteristics according to vitamin D3 level.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Vitamin D3 level</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Insufficiency</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Age of women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29 years</td>
<td>9</td>
<td>20.0</td>
</tr>
<tr>
<td>30-39 years</td>
<td>30</td>
<td>66.7</td>
</tr>
<tr>
<td>≥40 years</td>
<td>6</td>
<td>13.3</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>38</td>
<td>84.4</td>
</tr>
<tr>
<td>Housewife</td>
<td>7</td>
<td>15.6</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>40</td>
<td>88.9</td>
</tr>
<tr>
<td>Abnormal</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>38</td>
<td>84.4</td>
</tr>
<tr>
<td>Rural</td>
<td>7</td>
<td>15.6</td>
</tr>
<tr>
<td>Clothing style</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veiled</td>
<td>8</td>
<td>17.8</td>
</tr>
<tr>
<td>Unveiled</td>
<td>37</td>
<td>82.2</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - 2</td>
<td>15</td>
<td>33.3</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>42.2</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>24.4</td>
</tr>
<tr>
<td>5 and more</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Antenatal care visits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>44</td>
<td>97.8</td>
</tr>
<tr>
<td>Irregular</td>
<td>1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Fisher’s exact test, **Chi-square test, *** One-way ANOVA.

A highly significant association was observed between neonates with low Apgar score at 1 and 5 minutes, low birth weight, small head circumference, respiratory distress syndrome (and maternal vitamin D3 deficiency (p<0.001). In addition, a highly significant association was observed between neonatal admission to NICU and maternal vitamin D3 deficiency (p<0.001) (Table 2).
Table 2: Distribution of neonatal characteristics according to vitamin D3 level.

<table>
<thead>
<tr>
<th>Neonatal characteristics</th>
<th>Vitamin D3 level</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Insufficiency</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>APGAR score at 1 minute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;7</td>
<td>44</td>
<td>97.8</td>
</tr>
<tr>
<td>&lt;7</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>APGAR score at 5 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;7</td>
<td>44</td>
<td>97.8</td>
</tr>
<tr>
<td>&lt;7</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>42</td>
<td>93.3</td>
</tr>
<tr>
<td>Low</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td>Head circumference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>42</td>
<td>93.3</td>
</tr>
<tr>
<td>Small</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>97.8</td>
</tr>
<tr>
<td>NICU admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>97.8</td>
</tr>
</tbody>
</table>

* Chi-square test, ** Fishers exact test.

Discussion

The present study showed that 31% of term pregnant women had vitamin D3 insufficiency and 24% had vitamin D3 deficiency. These findings were lower than the findings of Al-Jebory et al. done in Iraq, which reported that 38% of term pregnant women had vitamin D3 insufficiency and 40% had vitamin D3 deficiency. Moreover, it is lower than another Iraqi study by Hilali et al., in which 50-65% of Iraqi women of childbearing age had vitamin D3 deficiency while 25% of them had vitamin D3 insufficiency.
The vitamin D3 findings in our study were lower than the results of Naseh et al. who found that 37% of pregnant women had vitamin D3 deficiency and 63% of pregnant women had vitamin D3 insufficiency in Iran.

Our study results were consistent with the results of Bassil et al. who reported 30.8% deficiency and 40% insufficiency. They also stated that vitamin D3 deficiency and insufficiency were prevalent in the Middle East countries, especially among children and women of childbearing age.

Vandevijvere et al. reported that 74.1% of women were vitamin D3 insufficient, 44.6% were vitamin D3 deficient and 12.1% were severely vitamin D3 deficient.

Choi et al. found that total prevalence of vitamin D3 deficiency among pregnant women in South Korea was 77.3%. Nageshu et al. found that 58.3% of pregnant women in India had vitamin D3 insufficiency and 13.8% pregnant women were deficient in vitamin D3.

The discrepancies in vitamin D3 prevalence between different countries have been attributed to differences in culture, nutritional habits, altitudes, sun exposure, and quality of antenatal care services. The deficiency and insufficiency of vitamin D3 are common worldwide. Many authors detected high prevalence of vitamin D3 deficiency in women, particularly during pregnancy and lactation periods.

Our study showed a highly significant association between pregnant women of younger age and vitamin D3 insufficiency (p<0.001), as 96.8% of pregnant women with vitamin D3 insufficiency were aged 20-29 years. This finding is consistent with the results of Ginde et al.’s study, which reported that adolescent and younger age pregnant women represented 95% women in the USA with vitamin D3 insufficiency. Fouda et al. carried out a study on women of childbearing age in Saudi Arabia and revealed that severe vitamin D deficiency was higher (92%) among younger age women. High prevalence of vitamin D3 deficiency in younger age pregnant women may be because women in their adolescence and younger age have a higher need for vitamin D for their growth and maturation, while pregnancy increases the burden and severity of vitamin D3 deficiency.

The current study revealed a highly significant association between housewives and vitamin D3 deficiency (95.8%; p<0.001). This is similar to the results of Bener et al.’s study done in Qatar who reported that pregnant housewives showed a higher prevalence of vitamin D3 deficiency and 88% of pregnant housewives had vitamin D3 deficiency. Dave et al. conducted a study on 110 pregnant women in India and documented that 98% of the pregnant housewives had a higher prevalence of vitamin D3 deficiency.

The strength of the present study was the prospective design while the main limitations of this study were inconsistent follow-up and a single-center study.

**Conclusion**

This study successfully showed that vitamin D3 deficiency among pregnant women can be directly linked with adverse neonatal outcomes like low Apgar score, low birth weight and head circumference, and respiratory distress syndrome. Monitoring the vitamin D3 levels can be beneficial both to the mother (pre and post pregnancy) and the newborn.

**Recommendations**

Emphasis on regular vitamin D and calcium monitoring of pregnant women during antenatal period and vitamin D supplementation campaigns for pregnant women should be encouraged.

Younger age, housewives, obese, rural residents, high parity and veiled pregnant women must be labeled as high-risk patients who require regular monitoring and vitamin D supplementations.

Further large nation-wide studies are needed to assess the relation between the effect of D3 deficiency on the mother and her own neonate.

**Conflicts of Interest:** None

**Source of funding:** Self

**Ethical Clearance:** Ethical clearance was taken from the scientific committee of the Iraqi Ministry of health and from Al-Eleiya Maternity Teaching hospital, Baghdad, Iraq
References


Caries Risk Assessment in Children Aged 6-12 years based on Parental Knowledge and Its Relationship with BMI and DMFT in Ahvaz

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Abstract

Background: The prevalence of dental caries in childhood is one of the problems related to children’s oral health. The aim of this study was to determine the risk of caries in children aged 6-12 years based on parental information and its relationship with BMI and DMFT in Ahvaz.

Methods and materials: The necessary permits were obtained from the Ahvaz Education Department, and then 300 samples of the city’s primary schools were selected. Students were examined with a disposable dental mirror and the number of decayed, missed, and restored permanent and deciduous teeth was recorded in the DMFT table. After that, the questionnaire was completed with the help of parents. The validity of this questionnaire was confirmed by 5 pediatric dentists and the reliability of this questionnaire was confirmed by conducting a pilot study on 10 children based on Cornbach coefficient of 0.85.

Results: Maternal occupation was associated with moderate caries risk (p = 0.007). Economic situation was associated with low risk (p = 0.0001). The mean weight of the child in the high risk group was significantly higher than the low risk groups (p <0.001) and medium risk (p <0.001). The mean DMFT in the low risk group was significantly lower than the medium risk (p <0.001) and high risk groups (p <0.001). The mean BMI in the high risk group was significantly higher than the medium risk groups (p <0.001) and low risk (p <0.001).

Conclusion: Economic status, mother’s job and child’s weight are associated with caries risk. Also, DMFT and BMI index have a direct and significant relationship with the risk of caries.

Keywords: Caries risk assessment (CRA), DMFT, BMI

Introduction

Tooth caries are the most common infectious disease in human societies.¹ Scientists have focused more on caries prevention and have achieved significant success in this regard.² One of the topics that has always been considered and questioned by researchers has been the issue of predicting the occurrence of caries using risk factors for this complication and great successes have been achieved.³ Given that caries is a multifactorial and complex disease; therefore, various researchers have tried to determine the susceptibility of people to tooth decay by examining each of the caries factors and their impact on the incidence of this complication, and thus predict future dental caries.⁴

The causing factors of caries including cariogenic microorganisms, fermentable carbohydrates, and susceptible hosts have been reported.⁵,⁶ In addition, ecologically, tooth decay, like other diseases, is due to

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an imbalance between the attacking agent that causes the primary lesion, and the inherent or acquired factor that changes the strength and susceptibility of the enamel. And the modifying factor that is present in the environment adjacent to the teeth, called plaque and saliva. Some types of foods and eating habits can increase the risk of weight gain and tooth decay in children. Therefore, nutritional patterns among obese children may be considered as a risk factor for obesity and dental caries.

Todays, there are two strategies for preventing disease: Whole population strategy and High risk strategy. Experience and research have shown that in the first type of prevention, most of the prevention costs that are spent on low risk people are actually wasted; so during the last two decades, the attention of prevention experts has been attracted to high risk strategy. Caries Risk Assessment (CRA), which is performed to identify people at risk of caries, has received a great deal of attention in research centers and dental schools over the past 20 years, and many dental schools around the world are performing this evaluation.

Performing CRA for all children, especially primary school children who are in mixed dentition period and need special care, can pave the way for faster achievement of prevention goals while identifying susceptible individuals. The aim of this study was to determine the risk of caries in children aged 6-12 years based on parental information and its relationship with BMI and DMFT in Ahvaz.

**Methods and Materials**

In the present descriptive epidemiological study, samples from primary schools in the city were selected by multi-stage cluster sampling method (300 people). Exclusion criteria of this study include unwillingness to cooperate, children with inherited and acquired underlying diseases.

Students were examined with a disposable dental mirror and the number of decayed, missed, and restored permanent and deciduous teeth was recorded in the DMFT Table 1. The DMFT index indicates the number of decayed permanent teeth (D), lost due to decay (M), and restored due to decay (F), and the higher the number, the worse the oral health and the lower the culture of prevention in society.

The questionnaire, which was written based on McDonald’s Children and Adolescents Dental Book 2016 and Pinkham 2019 was completed with the help of parents. The validity of this questionnaire was confirmed by 5 pediatric dentists and the reliability of this questionnaire was confirmed by conducting a pilot study on 10 children based on Cornbach coefficient of 0.85.

The questionnaire consists of 2 parts:

1- Demographic and background information questionnaire that includes questions about age, parents ‘education, parents’ occupation, economic status, birth rank, number of visits to the dentist, oral hygiene, fluoride consumption, consumption of different types Sweets and dates of the last filled teeth

2- The second part of the questionnaire was prepared based on the guidelines of the American Association of Pediatric Dentists to assess the risk of caries. This guide consists of three main sections, which include biological, protective, and clinical findings.

This study has been approved by the ethics committee of Ahwaz University of Medical Sciences. The study process was explained to all parents and informed consent was obtained from them.

After data collection, SPSS software version 20 and analysis of variance ANOVA and Tukey, chi square were used to analyze the data. Values of p <0.05 were considered as significant levels.

**Results**

There was no significant difference in caries risk (p <0.05) with the variables of Parent’s education, father’s occupation, gender, number of siblings, immigration, Seat belt, low birth weight, high birth weight, comorbidities, drug use, child age, mother age, father age, child height. Maternal occupation was associated with moderate caries risk (p = 0.007). Economic situation was associated with low risk (p = 0.0001). The mean weight of the child in the high risk group was significantly higher than the low risk groups (p <0.001) and medium risk (p <0.001). The mean weight of the child was not significantly different between the two groups of moderate and low risk (p = 0.548) (Table 1 and Figure 1).
Table 1. Description and comparison of the frequency/ mean of the demographic variables of the subjects based on the risk groups.

<table>
<thead>
<tr>
<th>Background variables</th>
<th>High risk group</th>
<th>Moderate risk group</th>
<th>Low risk group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency(%) / mean±SD</td>
<td>Frequency(%) / mean±SD</td>
<td>Frequency(%) / mean±SD</td>
<td></td>
</tr>
<tr>
<td>Total Number</td>
<td>164</td>
<td>104</td>
<td>32</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Mother’s education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under diploma</td>
<td>46(28%)</td>
<td>33(31.7%)</td>
<td>12(37.5%)</td>
<td>0.345NS</td>
</tr>
<tr>
<td>Diploma</td>
<td>62(37.8%)</td>
<td>39(37.5%)</td>
<td>15(46.9%)</td>
<td></td>
</tr>
<tr>
<td>university</td>
<td>56(34.1%)</td>
<td>32(30.8%)</td>
<td>5(15.6%)</td>
<td></td>
</tr>
<tr>
<td>Father’s education</td>
<td></td>
<td></td>
<td></td>
<td>0.163NS</td>
</tr>
<tr>
<td>Under diploma</td>
<td>51(31.1%)</td>
<td>33(31.7%)</td>
<td>13(40.6%)</td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>56(34.1%)</td>
<td>45(43.3%)</td>
<td>7(21.9%)</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>57(34.8%)</td>
<td>26(25%)</td>
<td>12(37.5%)</td>
<td></td>
</tr>
<tr>
<td>Mother’s job</td>
<td></td>
<td></td>
<td></td>
<td>0.007*</td>
</tr>
<tr>
<td>Housewife</td>
<td>62(37.8%)</td>
<td>19(18.3%)</td>
<td>13(40.6%)</td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>53(32.3%)</td>
<td>48(46.2%)</td>
<td>8(25%)</td>
<td></td>
</tr>
<tr>
<td>Self-employed</td>
<td>49(29.9%)</td>
<td>37(35.6%)</td>
<td>11(34.4%)</td>
<td></td>
</tr>
<tr>
<td>Father’s job</td>
<td></td>
<td></td>
<td></td>
<td>0.432NS</td>
</tr>
<tr>
<td>Employee</td>
<td>55(33.5%)</td>
<td>30(28.8%)</td>
<td>9(28.1%)</td>
<td></td>
</tr>
<tr>
<td>Self-employed</td>
<td>59(30.5%)</td>
<td>34(32.7%)</td>
<td>15(46.9%)</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>50(30.5%)</td>
<td>40(38.5%)</td>
<td>8(25%)</td>
<td></td>
</tr>
<tr>
<td>Economic status</td>
<td></td>
<td></td>
<td></td>
<td>0.0001*</td>
</tr>
<tr>
<td>Below 2.5</td>
<td>24(14.6%)</td>
<td>0(0%)</td>
<td>2(6.3%)</td>
<td></td>
</tr>
<tr>
<td>2.5-5</td>
<td>105(64%)</td>
<td>92(88.5%)</td>
<td>14(43.8%)</td>
<td></td>
</tr>
<tr>
<td>Above 5</td>
<td>35(21.3%)</td>
<td>12(11.5%)</td>
<td>16(50%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>0.15NS</td>
</tr>
<tr>
<td>male</td>
<td>81(49.4%)</td>
<td>40(38.5%)</td>
<td>17(53.1%)</td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>83(50.6%)</td>
<td>64(61.5%)</td>
<td>15(46.9%)</td>
<td></td>
</tr>
<tr>
<td>Siblings number</td>
<td></td>
<td></td>
<td></td>
<td>0.965NS</td>
</tr>
<tr>
<td>1</td>
<td>46(28%)</td>
<td>27(26%)</td>
<td>11(34.4%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>42(25.6%)</td>
<td>28(26.9%)</td>
<td>7(21.9%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>36(22%)</td>
<td>25(24%)</td>
<td>8(25%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40(24.4%)</td>
<td>24(23.1%)</td>
<td>6(18.8%)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1. Description and comparison of the frequency/mean of the demographic variables of the subjects based on the risk groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low risk</th>
<th>Moderate risk</th>
<th>High risk</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration</td>
<td>yes</td>
<td>13(7.9%)</td>
<td>4(3.8%)</td>
<td>5(15.6%)</td>
<td>0.075NS</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>158(96.3%)</td>
<td>101(97.1%)</td>
<td>32(100%)</td>
<td>0.538NS</td>
</tr>
<tr>
<td>Seat belt</td>
<td>yes</td>
<td>70(42.7%)</td>
<td>51(49%)</td>
<td>16(50%)</td>
<td>0.52NS</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>158(96.3%)</td>
<td>101(97.1%)</td>
<td>32(100%)</td>
<td></td>
</tr>
<tr>
<td>Premature birth</td>
<td>yes</td>
<td>6(3.7%)</td>
<td>3(2.9%)</td>
<td>0(0%)</td>
<td>0.538NS</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>158(96.3%)</td>
<td>101(97.1%)</td>
<td>32(100%)</td>
<td></td>
</tr>
<tr>
<td>Underweight at birth</td>
<td>yes</td>
<td>11(6.7%)</td>
<td>7(6.7%)</td>
<td>0(0%)</td>
<td>0.319NS</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>153(93.3%)</td>
<td>97(93.3%)</td>
<td>32(100%)</td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td>yes</td>
<td>6(3.7%)</td>
<td>4(3.8%)</td>
<td>4(12.5%)</td>
<td>0.084NS</td>
</tr>
<tr>
<td></td>
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<td>158(96.3%)</td>
<td>100(96.2%)</td>
<td>28(87.5%)</td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>yes</td>
<td>4(2.4%)</td>
<td>2(1.9%)</td>
<td>3(9.4%)</td>
<td>0.080NS</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>160(97.6%)</td>
<td>102(98.1%)</td>
<td>29(90.6%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Low risk</th>
<th>Moderate risk</th>
<th>High risk</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child age</td>
<td>8.99±2.02</td>
<td>8.83±2.02</td>
<td>9.56±1.89</td>
<td>0.195NS</td>
<td></td>
</tr>
<tr>
<td>Mother age</td>
<td>32.99±1.89</td>
<td>33.22±1.69</td>
<td>32.84±1.74</td>
<td>0.463NS</td>
<td></td>
</tr>
<tr>
<td>Father age</td>
<td>39.29±1.75</td>
<td>38.87±1.61</td>
<td>39.34±1.70</td>
<td>0.113NS</td>
<td></td>
</tr>
<tr>
<td>Child height</td>
<td>134.55±3.21</td>
<td>135.13±3.03</td>
<td>135.72±3.47</td>
<td>0.1NS</td>
<td></td>
</tr>
<tr>
<td>Child weight</td>
<td>29.77±4.41</td>
<td>26.16±2.48</td>
<td>26.91±3.33</td>
<td>0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

Fluoride levels, fluoride toothpaste, fluoride treatment, dentist, toothache, overnight breastfeeding, Teeth sucking, Tooth hurt, Timely growth, Tooth disorder, Decay treat ex, bruxism, Sugar drink food, Clean tooth gum, Brushing supervision Tooth brushing, Untreated mom, Untreated dad, Child tooth decay, Brushing together and Common dishes did not differ significantly in different caries risk groups (p < 0.05). The mean DMFT in the low risk group was significantly lower than the medium risk (p < 0.001) and high risk groups (p < 0.001). The mean DMFT level was not significantly different between the moderate and low risk groups (p = 0.869).

The mean BMI in the high risk group was significantly higher than the medium risk (p < 0.001) and low risk groups (p < 0.001). The mean BMI was not significantly different between moderate and low risk groups (p = 0.735).
Discussion

The issue of caries prediction over the past two decades has been the focus of researchers and experts in prevention. The average DMFT increases with the risk of caries; so by being low risk, this index has a lower number than being the medium and high risk; so the higher DMFT in patients with high caries risk indicates a direct and effective relationship between environmental factors and dental caries. The findings of the present study showed that economic status, maternal occupation and child weight are associated with low, moderate and high caries risk status. Along with the present study, in the study of Khodakarami et al., the level of education of mothers had a significant relationship with students’ attitudes toward oral health. In the study of Shirazi et al., The mother’s education had significant effects on dental plaque index. According to a study by Farsi et al., Previous caries, enamel demineralization, and socioeconomic status are important risk factors for caries. Although Pourhashemi in his study did not find a significant relationship between children’s dental health in the study area with occupation and level of education, but in the present study similar to the studies conducted in Iran and other countries, a significant relationship was found between the variable of maternal education and caries risk.

In the study of Pinto et al, the research on children, no significant relationship was shown between tooth decay and their weight. Also, Kopycka-Kedziersawski et al, did not report a significant relationship between tooth decay and the risk of overweight in American children aged 2-18 years. In a systematic study, Kantovitz et al identified only one study that had sufficient evidence to determine the association between weight and dental caries. However, in the present study, it was found that with increasing BMI, the risk of caries also increases, so that in the high risk group, this rate is higher than the average and low risk groups.

On the contrary, the study of Yousefi et al acknowledged that the dmft + DMFT index has an inverse and significant relationship with the body mass index of children. In the study of Pourhashemi et al, there was no clear relationship between body mass index
and childhood caries; in some cases, with increasing body mass index, the frequency of caries increased and in some cases decreased.\textsuperscript{20} In a study by Sheller et al, examining the relationship between body mass index and childhood caries in the United States, there was no association between dmft index values and the number of teeth with pulp involvement or BMI grouping, which is inconsistent with the findings of the present study.\textsuperscript{21} In another study in German primary schools, a significant and direct relationship was reported between body mass index and the incidence of dental caries in their primary and permanent teeth, which is consistent with the present study.\textsuperscript{22} Gerdin et al also reported a significant relationship between dental caries and Swedish children’s BMI.\textsuperscript{27}

Most of studies focused on different communities. Also, other criteria for body fat distribution such as waist circumference and waist to hip ratio have not been evaluated in this study. Obesity and overweight depend on many genetic and environmental factors.\textsuperscript{28,29} Obviously, it is not possible to differentiate and detect the effects of these factors in a cross-sectional study such as the present study; therefore, comparison is not possible. However, it may be assumed that depending on the society, children who are highly prone to obesity and the role of environmental factors is important in their obesity, may be at risk of caries due to poor eating habits and carbohydrate intake.

Finally, without considering genetic factors, some environmental factors are associated with the occurrence of caries and it is possible to assess the risk of caries with the help of these factors; But more articles and research are needed in this area.

**Conclusion**

It can be concluded:

1. Some environmental risk factors such as economic status, mother’s job and child’s weight are associated with caries risk.

2. The DMFT index increases with increasing caries risk, so by being low risk, this index has a lower number than the medium and high risk.

3. BMI is directly and significantly related to the risk of caries.

**Conflict of Interest:** Authors have declared that no competing interests exist.

**Ethical Clearance:** Ethical clearance taken from ethical committee of Ahvaz Jundishapur University of Medical Sciences.

**Funding:** None

**References**


5. Dean JA. McDonald and Avery’s Dentistry for the Child and Adolescent-E-Book: Elsevier Health Sciences; 2015.


Perianal Care Proven to Reduce the Incidence of Diaper Dermatitis

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Maintaining a clean perianal area is an attempt to prevent and treat diaper dermatitis. The purpose of cleaning the perianal area is to prevent irritation. The practice of cleaning the perianal area is done by considering the physiology of the skin such as, normal flora, bacteria, and skin pH. Cleaning the perianal area can use water and a soft cloth without rubbing the skin hard. The purpose of applying evidence based in nursing practice is to identify the effect of perianal care by using water against diaper dermatitis with skin integrity issues. Search evidence of nursing practice used the PICO analysis. Thirty-four toddlers were divided into two groups: the intervention group (n = 17) and the control group (n = 17). The analysis used was Wilcoxon, and Mann-Whitney test. There was no significant difference in diaper dermatitis score in the intervention group and control group (ρ> 0.05). Cleaning the perianal area using water and tissues can be used to prevent and treat diaper dermatitis.

Keyword: diaper dermatitis, skin integrity, perianal care

Introduction

Diaper Dermatitis (diaper rash, napkin dermatitis, nappy rash) is an acute inflammatory disorder of the skin of baby - that is common in babies and children/toddlers of 1 - 3 years old.1 Diaper dermatitis is a non-immunological response to skin irritants that cause skin cell hydration disorders.2 Diaper dermatitis can occur in patients who use diapers and patients who experience incontinence. The highest incidence of diaper dermatitis occurs at the age of 9 to 12 months.1,3 Diaper dermatitis is common in children, but this condition can cause pain in children and cause problems for their parents or caregivers - some of which are itching sensation, discomfort, and pain that causes the child to be restless and fussy. Diaper dermatitis is easy to treat with good skin care, but if diaper dermatitis is not treated, it can result in worse conditions such as pain, bacterial and fungal infections, and erosive Jacquet’s dermatitis.4

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The prevention and treatment management are very important for patients. Cleaning the perianal area with water is an independent action that nurses can do - by involving parents - to prevent and reduce the incidence of diaper dermatitis. The use of water to clean the perianal area is an effective intervention that does not require large costs and does not cause irritation. This innovation project aimed to determine the effectiveness of cleaning the perianal area using water against diaper dermatitis in toddlers with diarrhea.

**Method**

This research was a scientific evidence-based nursing care application or Evidence Base Nursing Practice using the Patient, Intervention Comparison, and Outcome (PICO) approach and literature studies through review of journals related to cleaning the perianal area to prevent and manage diaper dermatitis. The PICO model is a method for identifying and formulating questions about a problem (Gardner, Kanaskie, Knehans, Salisbury, & Doheny, 2016).

Based on the PICO model, the following problems identified in the implementation of the EBNP are: 1) Patients: 34 patients under five who experience diarrhea. 2) Intervention: The intervention in implementing the EBNP is to clean the perianal area with water. 3) Comparison: Cleaning the perianal area with wet tissues. 4) Result: Cleaning the perianal area with clean water is proven to reduce and prevent diaper dermatitis. The results of applying/implementing [the intervention on]

---

**Results**

The results on the characteristics of the patient respondents in the application/implementation of EBNP of cleaning the perianal area to reduce the incidence of diaper dermatitis that was carried out on 34 respondents who were divided into two groups, namely the control group and the intervention group - are as follows:

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>Wet Tissue (n = 15)</th>
<th>Water (n = 15)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n   %</td>
<td>n   %</td>
<td>n   %</td>
</tr>
<tr>
<td>1</td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baby</td>
<td>15   88.3</td>
<td>9  52.9</td>
<td>24  70.9</td>
</tr>
<tr>
<td></td>
<td>Toddler</td>
<td>2   11.7</td>
<td>6  35.3</td>
<td>8  23.5</td>
</tr>
</tbody>
</table>

---
Table 1. The Distribution of Respondent Characteristics by Age, Type of Diapers, and Use of Barriers in Children’s Infection Rooms in February-April 2017 (n = 34)

<table>
<thead>
<tr>
<th>Preschool Children</th>
<th>0</th>
<th>0</th>
<th>2</th>
<th>11.7</th>
<th>2</th>
<th>5.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>17</td>
<td>100</td>
<td>17</td>
<td>100</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaper Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorbent</td>
<td>17</td>
<td>100</td>
<td>17</td>
<td>100</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>Non Absorbent</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100</td>
<td>17</td>
<td>100</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of Barriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>47.1</td>
<td>6</td>
<td>35.3</td>
<td>14</td>
<td>41.1</td>
</tr>
<tr>
<td>Not</td>
<td>9</td>
<td>52.9</td>
<td>11</td>
<td>64.7</td>
<td>20</td>
<td>58.9</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100</td>
<td>17</td>
<td>100</td>
<td>34</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3.1 shows that in the control group most of the respondents are in the baby age category, with 15 respondents (88.3%); similarly, the intervention group is mostly consisted of most of respondents in the baby age category, with 9 respondents (52.9%). Based on the type of diaper, the respondents in both groups are 100% using the absorbent diaper type. Based on the use of barriers, the respondents in the control group mostly do not use barrier, with 9 respondents (52.9%), similarly, in the intervention group most of the respondents (11 people (64.7%)) also do not use a barrier.

The analysis of differences in diaper dermatitis scores in the intervention group and the control group was aimed to analyze the diaper dermatitis scores before and after the action. The homogeneity test used was the Levine test; the test results show that the variables of age, diaper type, and barrier use have the same variants in the two groups. Meanwhile, the data normality test used was the Shaphiro-Wilk analysis. The results of the data normality test show that the diaper dermatitis score - both before and after the intervention - have a p value of < 0.05, which means that the data in this research were not normally distributed. Therefore, for data that were not normally distributed, the type of statistical test used was the non-parametric test, namely Wilcoxon test to test a paired comparison between two groups.
Based on Figure 1, it can be seen that the decrease in diaper dermatitis scores in the intervention group is better than in the control group.

**Table 2. The Analysis of Difference in Diaper Dermatitis Score between the Intervention and Control Groups in the Second, Third and Fourth Days (n = 34)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaper dermatitis score</td>
<td>Intervention Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.179</td>
</tr>
</tbody>
</table>

Based on table 2, it can be seen that there is no significant difference in the difference in diaper dermatitis scores between the intervention group and the control group (p > 0.05).

**Discussion**

Damage to skin integrity often occurs in children who suffer from diarrhea. Due to the frequent defecation, the area of the skin around the perianal is more often exposed to feces, resulting in irritation and redness of the skin. Feces can cause skin irritation. Bacterial enzymes present in feces reduce urea and release ammonia. This causes an increase in pH and activates fecal proteases and lipases. This enzyme causes erythema, damages skin integrity, and increases the incidence of diaper dermatitis. Diarrhea is one of the causes of diaper dermatitis. Another study also explains that diarrhea is a significant factor that increases the risk of diaper dermatitis in children aged 1-24 months.

Diarrhea is a disease that can cause injury or damage to the skin. Injuries to the skin or the presence of damage to the skin can be considered as indicators of the quality of care in an acute care setting. Patients who use diapers and experience diarrhea are at risk of experiencing damage to skin integrity in the perianal area - or what is often called as diaper dermatitis. Preventing damage to the skin requires proper procedures. Efforts that can be made to prevent and manage patients with diaper dermatitis - one of which is by cleaning the perianal area regularly with water. After defecating, rinse the perianal area gently with water without rubbing it hard.

There are many variations that can be used to clean the perianal area, such as: cleaning with soap and water, cleaning with disposable or wet tissue, and cleaning
with the traditional way which uses water. Nowadays, many parents prefer to use tissue to clean the perianal area of their babies/children. A study on 117 children aged 6-24 months which was aimed to investigate the effectiveness of tissue and water [in cleaning the perianal area] against the incidence of diaper dermatitis, reveals that no significant is found in the difference of diaper dermatitis severity between the tissue and water groups. However, few diaper dermatitis in the area of *candidiasis intertriginosa* is found in patients who used tissue.\(^5\)

Similarly, based on the results of the innovation project implementing the EBNP, it is found that there is no significant difference between cleaning the perianal area using water and tissue (\(p > 0.05\)). The results of this research are in accordance with the research by Adalat, which concludes that there is no significant relationship between the use of tissue, water, or a combination of both. Another similar study also shows that there is no significant difference in skin hydration in the two groups, although there is mild dermatitis found in babies who are cleaned using cotton and water.\(^13\)

A study has suggested that there is a significant relationship between perianal skin care and the risk of damage to perianal skin integrity in babies with diarrhea. Perianal skin care carried out in this study was by using water and cotton to clean the perianal area. This result indicates that the risk of damage to skin integrity in the perianal area of children/toddlers with diarrhea and receive skin care according to practice standards is lower than in children/toddlers with diarrhea and receive skin care according to hospital habits.

This study reveals that there is not enough evidence to support or reject the use of water to clean the perianal area to prevent and treat diaper dermatitis in children under five. This can be caused by several factors, among others, the inadequate number of respondents, the age of the respondents, the use of barriers, and the type of diaper.

In this implementation of the EBNP, the results show that the majority of respondents’ ages are in the baby age category. This is consistent, that the peak incidence of diaper dermatitis is between the ages of 9 and 12 months. The highest prevalence of diaper dermatitis occurs in babies aged 9 to 12 months; this may be due to the introduction of solid foods during this period which causes high frequency of bowel movements and urination in some babies. Breastfeeding also plays an important role in the prevention of diaper dermatitis because the feces of exclusively breastfed babies have a lower pH and protease and lipase activity.\(^14\)

Another factor that can affect the incidence of diaper dermatitis is the use of diapers. Most of the respondents in this EBNP implementation research use an absorbent type diaper. Indeed, in order to prevent diaper dermatitis, experts recommend minimizing the use of diapers and if you use diapers [for your babies] choose the disposable diapers and you should change [your babies’] diapers more frequently.\(^14\) The use of disposable diapers is recommended because they contain absorbent components, such as hydrocellulose gel, which is very good for preventing moist conditions in skin.\(^15\) Disposable diapers with super-absorbent gel, an external porous cloth layer, and an absorbent outer layer have been shown to reduce the incidence of diaper dermatitis.

Besides the use of diapers, the use of skin barriers is another factor that can cause the incidence of dermatitis. Based on the results of this implementation of the EBNP, it is found that some respondents use a barrier as an effort to prevent diaper dermatitis. Ersoy-Evans, Akinci, Dogan, and Atakan explain that the use of skin barriers such as creams is an important method to prevent and treat diaper dermatitis. Besides preventing contact between skin and urine and feces, creams can improve the healing of diaper dermatitis, especially in babies. These creams usually contain zinc oxide, petrolatum jelly, cod liver oil, dimethicone, and dexpanthenol which can cure diaper dermatitis.

A study found that water is not statistically significant for cleaning perianal area but clinically water is shown to reduce the diaper dermatitis score when it is compared to using a tissue (wet tissue). Wet tissue can cause complications, especially because of the presence of preservatives such as methylisothiazolinone. The use of wet tissue (baby wipes) to clean the perianal area is as light as using water where there is no change in skin condition. But wet tissue contains preservatives and fragrances which have the potential to be sensitized topically, thus using wet tissue is not recommended if
damage to skin integrity has occurred.\textsuperscript{11}

Finally, using water to clean the perianal area is an effective intervention that does not require large costs and does not cause irritation. Nurses must be able to identify risk factors, preventive measures, and treatments that will be given to prevent diaper dermatitis – one of which is by regularly cleaning the perianal area.\textsuperscript{14}

**Conclusion**

There is no significant difference in the dermatitis diaper scores after being given perianal care – both in the intervention group and the control group. However, the use of water has been clinically proven to lower the diaper dermatitis score when compared to the use of wet tissue.

**Source of Fund :** Self

**Ethical Approval :** Sekolah Tinggi Ilmu Kesehatan William Booth Surabaya Tahun 2019

**Conflict of Interest :** Nil

**References**

LIP Prints Patterns: A Study among the Aryan and Dravidian Ethnic Population in India- A Cross Sectional Study

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Abstract

Background: Lip prints are subjected to be unique for all the individuals except monozygotic twins; hence they can be an important indicator for human identification. Since, Aryan population are descendants of north India and Dravidian population are descendants of south India. Aim: The aim of the study was to assess the uniqueness of lip prints among Aryan and Dravidian ethnic population. Materials and method: A cross sectional study was conducted among 192 individuals of Aryan and Dravidian population. Convenient sampling was used to select the study population among the Tamilnadu and Chandigarh states in India. The lip prints were recorded with the help of lip sticks and glue portion of the cello tape. Statistical analysis was done using spss software version 20.0. Results: In the present study, branched lip print pattern was predominantly seen in males of Dravidian population. In contrast, least commonly predominant was partially vertical pattern which was absent in both the males and females of Aryan population. Conclusion: Lip prints are a unique feature for human identification. In this study, it was found that branched lip print pattern was higher in males of Dravidian population and the partially vertical type was least predominant among the males and females of Aryan and Dravidian population.

Keywords: Lip prints, Aryan, Dravidian, ethnic population, males, females.

Introduction

Lip prints vary from person to person and are used as a diagnostic tool in forensic odontology to augment human identification criteria. The classification of Lip prints is based on lip wrinkles and lip grooves. The lip-print pattern has been used as a tool for determination of gender, geographic origin, the number of people who created a mark, forensic investigation, detection of tactical and criminalist information. Cheiloscopy is a term subject to the patterns of wrinkles and grooves present on the labial mucosa. R.fischer was the first person to notice the biological phenomenon present in the red part of labial mucosa. Followed by in 1932, Edmond Locard a France criminologist identified the use of lip print for identification of person and in 1950 Le Moyne Snyder proved in a criminal case that lip print serves as an important identification to find out the person. Tsuchihasi in Japan studied about the lip print impression which led to the classification of lip print in 1974. Lip prints can be recorded in number of ways which includes the photography method, applying the lipstick on labial mucosa and recording the lip print pattern, using a finger printer, detecting the lip print using magna brush or magnetic powder. The various pigments in the labial mucosa can be analysed by using thin layer chromatography and Ultraviolet light. In dentistry, Lip prints can be used for comparison of homicide cases where the victims do not have teeth or readily available dental records for easy identification of the victim. According to the literature, prevalence

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of dental abnormalities was reported in Dravidian population was 31.5% in which 6.6% of people had microodontia\(^1\). The dental disease more common among the Dravidian population was malocclusion\(^2\) and periodontitis\(^3\). There was no enough literature in the field of dentistry to support the same among the Aryan population. Further knowing the importance of lip prints in the field of dentistry, the common lip print pattern among two different populations existing in India was a question of concern. The gene most predominant in Aryan population was R1A1 and the gene predominant in Dravidian population is still in question. Since the north Indians are descendants of Aryan population and the south Indians are descendants of Dravidian population, the objective of the study is to analyse the uniqueness in lip prints among the Aryan and Dravidian ethnic population in India.

**Materials and Method**

A cross sectional study was conducted to assess the uniqueness of lip print pattern among the Aryan and Dravidian population. Decision tree methodology was designed before conducting the study. Since Aryan was predominant in Chandigarh while Dravidian population lived in large amounts in Tamilnadu, the data was collected from respective states of India. Ethical clearance was obtained from the Department of Public Health Dentistry, SRM dental college and hospital, Ramapuram. The study was conducted in the month of January 2020. Convenient sampling technique was used and data samples were obtained. Subjects without any abnormality in the lips were included in the study based on their willingness to participate. The exclusion criteria were the individual whose parents or grandparents had a history of inter-caste marriage were excluded from the study along with individuals having cleft lip, individual with any inflammation, individual allergic to the lip stick. According to Suzuki and tsuchihasi 1974 classification of lip prints was categorized as vertical, partially vertical, branched, intersected and reticular. The individual were made to sit relaxed and the lips were cleaned with cotton and a thin layer of dark red colour lip was applied in the lips and they were asked to spread uniformly. The glue portion of the cello tape was stuck into the surface of the lip under firm pressure so that the cello tape firmly sticks to the lip surface. Two duplicate prints were taken to avoid improper recording of lip print. Consecutively, the lip prints were analysed and verified by the experts for whom the Intra reliability was calculated using kappa statistics was found to be 0.89. Descriptive statistics was done to find out the overall number and percentage of study sample distribution among the Aryan and Dravidian population and gender distribution was also analysed using spss software version 20.0. Inferential statistics was performed by using Chi square to find out association of lip print pattern between the male and female and p value <0.05 was found to be statistically significant.

**Results**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Lip print pattern</th>
<th>Aryan population</th>
<th>Dravidian population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of males</td>
<td>Percentage of males</td>
</tr>
<tr>
<td>1</td>
<td>Branched</td>
<td>15</td>
<td>14.9%</td>
</tr>
<tr>
<td>2</td>
<td>Intersected</td>
<td>3</td>
<td>2.9%</td>
</tr>
<tr>
<td>3</td>
<td>Partially vertical</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>Reticular</td>
<td>8</td>
<td>9.5%</td>
</tr>
<tr>
<td>5</td>
<td>Vertical</td>
<td>13</td>
<td>12.6%</td>
</tr>
</tbody>
</table>

Table 1 shows the total number and total percentage of males in both ethnic population and found that higher percentage of branched lip print is found among the males of Dravidian population and no partially vertical lip print was found in males of Aryan population.
Table 2: Comparison of lip print pattern among the females of Aryan and Dravidian population

<table>
<thead>
<tr>
<th>S.No</th>
<th>Lip print pattern</th>
<th>Aryan population</th>
<th>Dravidian population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of female</td>
<td>Percentage of females</td>
</tr>
<tr>
<td>1</td>
<td>Branched</td>
<td>16</td>
<td>17.5%</td>
</tr>
<tr>
<td>2</td>
<td>Intersected</td>
<td>3</td>
<td>3.1%</td>
</tr>
<tr>
<td>3</td>
<td>Partially vertical</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>Reticular</td>
<td>7</td>
<td>7.5%</td>
</tr>
<tr>
<td>5</td>
<td>Vertical</td>
<td>7</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

Table 2 shows the total number and total percentage of females in both ethnic population and found a higher percentage of vertical lip print pattern among the females of Dravidian and no partially vertical lip print pattern was found among the females of Aryan population.

Table 3: Comparison of lip print pattern among the males and females of Aryan population

<table>
<thead>
<tr>
<th>S.No</th>
<th>Lip print pattern</th>
<th>Aryan population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of male</td>
</tr>
<tr>
<td>1</td>
<td>Branched</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Intersected</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Partially vertical</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Reticular</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Vertical</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 3 shows the lip print pattern among the males and female of Aryan population found that branched lip print was higher in percentage among the Aryan population and no partially vertical lip print pattern was found.
Table 4: Comparison of lip print pattern among the males and females of Dravidian population

<table>
<thead>
<tr>
<th>S.No</th>
<th>Lip print pattern</th>
<th>Dravidian population</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of male</td>
<td>Percentage of male</td>
<td>Number of female</td>
<td>Percentage of female</td>
<td>Total number</td>
</tr>
<tr>
<td>1</td>
<td>Branched</td>
<td>28</td>
<td>23.7%</td>
<td>20</td>
<td>16.6%</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>Intersected</td>
<td>4</td>
<td>3.3%</td>
<td>4</td>
<td>3.3%</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Partially vertical</td>
<td>3</td>
<td>2.5%</td>
<td>1</td>
<td>0.8%</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Reticular</td>
<td>8</td>
<td>6.6%</td>
<td>8</td>
<td>6.6%</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Vertical</td>
<td>19</td>
<td>15.8%</td>
<td>25</td>
<td>20.8%</td>
<td>44</td>
</tr>
</tbody>
</table>

Table 4 shows the lip print pattern among the males and female of Dravidian population and found a higher percentage of branched lip print pattern was found followed by vertical lip print and least common was partially vertical lip print pattern among the Dravidian population.

Table 5: Association between the lip print pattern and gender difference between the study populations

<table>
<thead>
<tr>
<th>S.No</th>
<th>Population</th>
<th>ARYAN</th>
<th>DRAVIDIAN</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of male</td>
<td>Percentage of male</td>
<td>Number of female</td>
</tr>
<tr>
<td>1</td>
<td>Branched</td>
<td>15</td>
<td>7.7%</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Intersected</td>
<td>3</td>
<td>1.5%</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Reticular</td>
<td>8</td>
<td>4.1%</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Vertical</td>
<td>13</td>
<td>6.6%</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 5 shows that association between the lip print pattern among the males and females of Aryan and Dravidian population. P value <0.05 was considered as statistically significant.
Table 6: Association between the lip print pattern among the Aryan and Dravidian population:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Population</th>
<th>ARYAN POPULATION</th>
<th>DRAVIDIAN POPULATION</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NUMBER OF POPULATION</td>
<td>PERCENTAGE OF POPULATION</td>
<td>NUMBER OF POPULATION</td>
</tr>
<tr>
<td>1</td>
<td>Branched</td>
<td>31</td>
<td>15.9%</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>Intersected</td>
<td>6</td>
<td>4%</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Reticular</td>
<td>15</td>
<td>7.7%</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Vertical</td>
<td>20</td>
<td>10.2%</td>
<td>44</td>
</tr>
</tbody>
</table>

Table 6 shows the association between the lip print pattern among the Aryan and Dravidian population and p value was calculated to know its statistical significance.

Discussion

Lip print is found to be unique from individual to individual except the monozygotic twins. Lip print always serves as an important diagnostic tool in finding out the age, demographic details and some important aspects in forensic odontology. Lip prints always remain as a standard approach in finding out many detective crimes and forensic branch always establish accurate ways for classification of lip furrows, lip grooves and wrinkles that constitute the human lip. The study was an attempt conducted to known the prominent lip print pattern seen among the Dravidian and Aryan ethnic population and found that an overall highest lip print pattern among the males and females of Aryan population was branched lip print pattern which was found to be 7.7% and 8.2%. In Dravidian population, branched lip print pattern was found to be highest among the males and vertical lip print pattern was found to be highest among the females which are found to be 14.3% and 12.8% which coincided with the study conducted by Venkatesh et al14 among the students of Karnataka and Bangalore. When comparative analysis was done between the Aryan and Dravidian population, it was found that highest lip print pattern was branched lip pattern reported among the males of Dravidian group which was 14.3% and lowest was recorded among females of Dravidian group which had partially vertical lip print pattern and one type of lip print pattern was not recorded among the males and females of Aryan group and also the males of Dravidian group which was portical lip print pattern which coincides with the study conducted by Prathibha Prasad et al15 among the Aryan-Dravidian population. Association between the gender difference and lip print among the Aryan and Dravidian population was assessed and p value was found to be statistically significant which was 0.011; the partially vertical type was not added in the analysis of p value since no record was found among the males and females of Aryan population. Association between the lip print pattern among the Aryan and Dravidian population was found to have a p value of 0.031 which was statistically significant. Sivapathasundharam et al study also concluded that branched lip print was common among the Indo-Dravidian population which coincided with our study which also gave a result that branched lip print was common among the study population. Limitation of the study was the sample size was not calculated according to confidence interval so reflection of the study cannot be applied to the generalised population.
Conclusion

Lip prints serving as a diagnostic tool to identify the human victims. The present study shows that branched lip print pattern was common and found to 15.9% among males and females of the Aryan population 22.5% in males and females among the Dravidian population. Second most common was the vertical lip pattern which was 10.2% and 13.3% among the Aryan and Dravidian population. Least common was partially vertical lip print pattern which was not found in Aryan population and 3.3% was reported among the Dravidian population.

Conflict of Interest: Nil

Source of Funding: Self

References

Detection of Endometrial TB in Patients with AUB using PCR Method of Assessment of Menstrual Blood Flow of Iraqi Females

Maad Mehdi Shallal,1 Farah Abdul Hussein Salih Al-Asadi2, Mohammad Ibrahim Mizaal3

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Abstract

Background: Iraq still considered one of the countries in the region with high prevalence of TB, there are about 20000 patients, accounts for 3% of the total number of cases, and the estimated death from TB exceeds 4000 per year. TB in Iraq until nowadays considered a health, public and social problem, and because of the wars and political problems, many programs, campaigns, and researches were hindered by this situation.

Objective: To detect endometrial Tuberculosis in women complaining from AUB (abnormal uterine bleeding) using real time PCR method of assessment via taking an endocervical swab.

Patients and methods: Prospective cross-sectional study carried at Department of obstetrics and gynecology in Baghdad teaching hospital /medical city over a period of eight months starting from October 2019, to August 2020. A total of 60 women complaining from abnormal uterine bleeding (AUB) who were scheduled to have endometrial biopsy for histopathology by dilatation and curettage (D&C), endocervical swab assessed for female genital tuberculosis (FGTB) by real time PCR.

Results: Out of 60 samples taken 6 were positive by real time PCR 10%, while there was only one case positive by histopathology 1.66% which was also positive by PCR the same patient gave history of pulmonary TB and received a nine month course of treatment, according to these findings the sensitivity of PCR found to be 100% while the specificity 92% and an accuracy of 92%.

Conclusion: Real time PCR considered one of the valuable diagnostic tools for mycobacterium tuberculosis, and because Iraq still showing high incidence of the disease, one should suspect mycobacterium tuberculosis in any women complaining from abnormal uterine bleeding and offer PCR in addition to other diagnostic modalities, luckily it will allow treatment at an early stage of the disease.

Keyword: Endometrium, TB, AUB, PCR

Introduction

Iraq still considered one of the countries in the region with high prevalence of TB, there are about 20000 patients, accounts for 3% of the total number of cases, the estimated death from TB exceeds 4000 per year (1).

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Phone number: +9647818069544.

TB in Iraq until nowadays considered a health, public and social problem, and because of the wars and political problems, many programs and campaigns and researches were hindered by this situation (2).

Tuberculous PID is largely limited to patients from developing countries. Pelvic infection usually occurs secondary to hematogenous spread from an extragenital source. Nevertheless, occasionally Mycobacterium tuberculosis can be transmitted sexually (3).
Genital TB occupy about 9% of extrapulmonary TB (4), TB endometritis occupy about 50-60% of genital TB and tends to be sporadic lesions whereas ulceration, caseous changes and bleeding seen later on to ends up by adhesion and amenorrhea (5). Diagnosis of genital TB suspected depending on patient’s symptoms with infertility being at the top of the list, then AUB whereas dyspareunia and dysmenorrhea at the bottom of the list (6).

One of the conventional methods used for diagnosis of tuberculosis is acid–fast bacilli staining which mainly relied on identification of *Mycobacterium tuberculosis* by microscopy or its growth on culture medium (7). Culture still considered a gold standard method in the diagnosis of genital TB with a low detection rate contributes to the paucity of mycobacteria in the genital tract and delay in the diagnosis due to slowly growing pathogen which requires about 3-6 weeks (8). histopathology required the presence of epithelioid granuloma in the examined tissue and because of the shedding of the endometrium there might be no time for the granuloma to be formed so the endometrial aspirate and endometrial sample will be negative and many cases will be missed for this reason (9).

PCR method are widely used nowadays, a rapid procedure requires few hours for the results to be obtained and can identify the nucleic acid sequence specific to mycobacterium tuberculosis and other mycobacteria in female genital TB, with a high detection rate <10 bacilli/ml including dead bacilli (10). Compared to acid–fast bacilli staining by Zeihl-Neelsen stain which needs 104-106bacilli/ml on the other hand culture requires 10-100bacilli/ml of tissue/fluid sample for the diagnosis (5). It is possible now to catch hidden genital tuberculosis by PCR, real time PCR noticeably decreases the false positive results because the amplification and detection takes place in the same reaction tube with a sensitivity reaches about 90-94% and specificity of 70-78% (11).

**Material and Methods**

Our prospective present study carried out in the department of obstetrics and gynaecology/ Baghdad teaching hospital, from October 2019 till August 2020, a total of 60 patients aged between 30-68 years attends the gynecology outpatient clinic complaining from abnormal uterine bleeding all were prepared for diagnostic dilatation and curetage(D&C) and endometrial sampling, after full history taking including any positive history for pulmonary TB and received a 9-month course of anti-TB drugs. after patient consent endocervical swab was taken before commencing D&C, the swab then kept in 1 ml of transport medium then diagnostic curetage was done and endometrial sample sent for histopathology after being fixed with formalin.

Swabs then sent for laboratory for processing by real time PCR using AmpliSens MTC-FRT PCR kit, mycobacterium tuberculosis detection by the PCR is based on the amplification of pathogen genome specific region using specific mycobacteria tuberculosis primers. AmpliSens MTC-FRT PCR kit is a qualitative test that contains the internal control (internal control STI-87) (12).

**Results**

Table 1 show that age group between (40-49) years was the main group (61.6%) with the mean age (47.3±5.6) years, blood group A+ represented (41.7%), parity between (4-6) was the main group, while Menorrhagia was the most common symptom (85.0%) and the (means ±SD) of endometrial thickness was (9.27±4.5) mm.
Table 1: Socio demographic characteristics of the studied group

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>40-49</td>
<td>37</td>
<td>61.6</td>
</tr>
<tr>
<td>50-59</td>
<td>19</td>
<td>31.7</td>
</tr>
<tr>
<td>≥60</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Age (Mean±SD) years**: 47.3±5.6

<table>
<thead>
<tr>
<th>Blood group</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>A+</td>
<td>25</td>
<td>41.7</td>
</tr>
<tr>
<td>AB+</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>B-</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>B+</td>
<td>7</td>
<td>11.6</td>
</tr>
<tr>
<td>O+</td>
<td>20</td>
<td>33.3</td>
</tr>
<tr>
<td>O-</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parity</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>1-3</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td>4-6</td>
<td>29</td>
<td>48.3</td>
</tr>
<tr>
<td>≥7</td>
<td>20</td>
<td>33.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menorrhagia</td>
<td>51</td>
<td>85.0</td>
</tr>
<tr>
<td>PMB</td>
<td>7</td>
<td>11.7</td>
</tr>
<tr>
<td>Metromenorrhagia</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Endometrial thickness (means±SD) mm of the studied group/mm**: 9.27±4.5
Figure 1 show that TB were positive in 6/60 (10.0%) of the patients and the rest 54/60 (90.0%) were negative by PCR test, while histopathological finding show that all patients 60 (100.0%) were TB negative.

![Graph](https://via.placeholder.com/150)

**Figure 2: Distribution of the studied group according to PCR test and histopathological finding**

As shown in table 2 all positive PCR were suffering from menorrhagia while no cases were found regarding other two symptoms (PMB and Metromenorrhagia). No significant difference was found between PCR outcome and symptoms of the disease (P≥0.05).

<table>
<thead>
<tr>
<th>Table 2: Relation between PCR and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Menorrhagia</strong></td>
</tr>
<tr>
<td>PCR +ve (n=6)</td>
</tr>
<tr>
<td>PCR -ve (n=54)</td>
</tr>
</tbody>
</table>

Ns= Not significant

Table 3 show that there is no significant association between endometrial thickness, parity and blood group with TB patients regarding PCR outcome. Moreover, no significant association between means of endometrial thickness and age with TB patients diagnosed by PCR (P≥0.05) (table 4).

<table>
<thead>
<tr>
<th>Table 3: Relation between different parameters and PCR outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endometrial thickness</strong></td>
</tr>
<tr>
<td>≤5mm</td>
</tr>
<tr>
<td>&gt;5mm</td>
</tr>
</tbody>
</table>
The validity results of histopathological findings regarding PCR test were sensitivity (0.0%), specificity (100%), +ve predictive value (0.0%), -ve predictive value (90.0%) and accuracy (90.0%). All these findings were shown in table 5.

Table 5: Validity test

<table>
<thead>
<tr>
<th>Validity test</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal (+ve)</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Normal</td>
<td>6 (10.0)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (10.0)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td>+ve predictive</td>
<td></td>
</tr>
<tr>
<td>-ve predictive</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
</tr>
</tbody>
</table>

Fisher exact test
Discussion

The incidence of FGTB differs worldwide; this is due to differences in studied groups, social stigma, the type of tests used to diagnose TB and its sensitivity and specificity, and the time at which the sample was taken with respect to the menstrual cycle. Although genital TB occurs secondary to pulmonary TB through hematogenous and lymphatic routes, primary genital TB can occur rarely when the male partner has genital TB such as TB epididymitis by transmission of infected semen.

Diagnosis of TB endometritis needs high index of suspicion because the disease is a symptomatic in majority of cases in addition to the paucity of the bacteria that’s why the diagnosis require a test with high sensitivity and specificity to detect the disease in early stages. (13, 14)

Depending on the fact that real time PCR has a high sensitivity and specificity in detecting mycobacterium tuberculosis and histopathology usefulness in detecting endometrial pathologies we conducted this study. In Histopathology the diagnosis of tuberculous endometritis based on the presence of epitheloid cell granulomas with or without Langerhans giant cells, and caseation necrosis which is seen on late disease (1).

In our study, out of 60 patients complaining from abnormal uterine bleeding in form of menorrhagia, metro menorrhagia and polymenorrhoea, endocervical swabs revealed that 6(10%) of those women were found to be positive for mycobacterium tuberculosis by real time PCR while histopathology detect only one case(1.7%) which is also positive by real time PCR and have positive history of pulmonary TB, the low detection rate of histopathology, in the current study, can be explained either to an early stage of the disease or the continuous shedding of the endometrium specially in our study when all the PCR positive women complain from menorrhagia, or due to presence of focal lesions which may be missed by curette. meaning that histopathology is false negative in 5 patients and thus give a sensitivity and specificity for real time PCR of 100% and 92% respectively.

These results when compared to study conducted by Malhorta et al (8), who reported an overall positivity by real time PCR alone 23.78% which is higher than in our study (10%) and a sensitivity of 94.28% which is approximate to current study.

Whereas Thangappah et al (9) study showed tuberculosis were positive in 36.7% by PCR and 6.9% for histopathology giving a lower sensitivity 57.1% for PCR and higher sensitivity for histopathology10.7% as compared to our study which was explained by Thangappah as cyclic shedding of the endometrium resulting in to poor formation of the granuloma and thus the endometrium will not show evidence of tuberculosis in all cycles so multiple specimens from different locations is advisable.

Our efforts in this study was to decrease the false positive cases, all the patients complain from abnormal uterine bleeding, have no clinical sign or symptoms of TB except one known case of pulmonary TB.

The other five out of six positive cases detected by PCR could be explained by early stages of the disease and the bacteria known to be indolent in genital tract or latent disease detected by PCR and the patient still without symptoms.

Other sources of false positivity could be due and the presence of dead bacillus or previous infection which was excluded by past medical history other causes could be the contamination which was overcome in this study by the type of PCR kit which includes the enzyme uracil-DNA glycosylase to reduce the risk of contamination, also the kit contains internal control (STI-87) used in the extraction procedure in order to control the extraction process of each sample individually and thus recognition of any reaction inhibition, furthermore the kit uses (hot-start) which greatly reduces the frequency of nonspecifically primed reaction (9)(12).

Conclusion

Real time PCR considered one of the valuable diagnostic tools for mycobacterium tuberculosis, and because Iraq still showing high incidence of the disease, one should suspect mycobacterium tuberculosis in any women complaining from abnormal uterine bleeding and offer PCR in addition to other diagnostic modalities, luckily it will allow treatment at an early stage of the disease.
No conflicts of Interest

Source of Funding: Self

Ethical Clearance: was taken from the scientific committee of the Iraqi Ministry of health

References


Nasal Polyps: An Etiological Analysis

Magda Abd El-latif1, Sayed Kadah2, Ahmed Yehia3

1Professor, ENT Department, Al-Azhar university, Cairo, Egypt, 2Assistant Lecturer, ENT department, Armed Forces College of Medicine

Abstract

Nasal polyps, the prolapsed linings of nasal mucosa are one of the commonly encountered conditions in the outpatient department of otolaryngology. The aim of this study is to study the etiology of nasal polyps and also to compare between the different methods used for diagnosis of nasal polyps.

This study was submitted prospectively on 100 patients diagnosed with nasal polyps (diagnosed either clinically or radiologically) with evidence of nasal obstruction, rhinorrhea and headache. From April 2015 till April 2017. All patients had been selected from the outpatient clinics of Al-Zahraa University Hospital and Kobry El Koppa military hospital. The study was approved by the ethics committee of Al Azhar Faculty of Medicine.

A total of 100 patients were analyzed, age range 18 to 56 yrs with a mean age of 38.5 yrs. There were 83 male and 17 female. The main presenting symptoms are nasal obstruction and rhinorrhea. Most of the cases was bilateral nasal polyps, The commonest clinical diagnoses were non-neoplastic simple nasal polyps 84% and about 13% were benign neoplastic, 3% were malignant. The commonest histological diagnosis among non-neoplastic nasal polyps was simple allergic nasal polyp, among benign neoplastic was inverted papilloma. Most of neoplastic lesions were presented by unilateral nasal polyps, where epistaxis was noticed mostly with vascular tumour and malignant ones. The results show that most of nasal polyps were simple non-neoplastic lesions especially bilateral ones, also for proper evaluation of nasal polyps clinical, radiological and histopathological evaluation should be done in all patients.

Keywords: nasal polyps, histopathology, inverted papilloma.

Introduction

Nasal polyps, the prolapsed linings of nasal mucosa are one of the commonly encountered conditions in the outpatient department of otolaryngology.

They are a common cause of nasal obstruction in the adult, while the diagnosis in children is so rare (0.1%). In the general population, the prevalence of NP is considered to be around 4%. It predominantly affects adults usually those older than 20. Cancers of the nose and paranasal sinuses account for less than 1% of all malignancies and about 3% of all head and neck cancers. It has a geographic tendency to affect the African, the Japanese, and the Arab. It is rarer in Western Europe and America. There are a variety of conditions ranging from benign lesions to malignant nasal tumors which may mimic simple nasal polyps.

It is impossible to determine clinically what pathology lies underneath. Therefore, nasal endoscopy, radiology and histopathology are employed to help us reaching the diagnosis.

The histopathological examination of the removed tissue helps to determine the actual pathology of the varied conditions labelled as nasal polyps.

The aim of the work:

The aim of this work was To study the etiology of nasal polyps and This will be reached by performing full nasal polyps examination pre and postoperative.

Patients and methods:

The study was approved by the ethics committee of Al Azhar faculty of medicine. The study was submitted
prospectively on 100 patients during the period from April 2015 till April 2018. All patients had been selected from the outpatient clinics of Al -Zahraa University Hospital and Kobri-El kobba military hospital. All patients underwent complete ear, nose and throat examination. Using the headlight and nasal endoscope, the nasal cavity was carefully examined for presence of nasal polyps.

The preoperative evaluation had included history with inquiry about the main complaint (nasal obstruction) and other nasal symptoms, (sneezing, rhinorrea , Post-nasal drip, Headache and nasal itching). Diagnostic nasal endoscopy was done.

CT Scan nose and paranasal sinuses and MRI was done in selected cases. Each patient had been managed by functional endoscopic sinus surgery. The procedure was done under general anesthesia followed by histopathology of the removed polyps.

**Data management and Analysis**

Quantitative data were shown as mean, SD, minimum and maximum. Qualitative data were expressed as frequency and percent. Chi- square test and Fisher exact test were used to measure association between qualitative variables as appropriate. P (probability) value was considered to be of statistical significance if it is less than 0.05.

**Results**

One hundred patients were included in the study, there were 83 males and 17 females, ranged in age between 18 and 56 years with a mean age of 38.5 years.

**Table 1. Gender and age distribution of the studied group**

<table>
<thead>
<tr>
<th>Studied group</th>
<th>N=100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Min-max</td>
<td>18-56</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>38.5±8</td>
</tr>
</tbody>
</table>

Nasal obstruction was the commonest symptom(100%) followed by nasal obstruction with rhinorrea(90%),smell disorder in 63%,headache was found in 82% and proptosis in 4% of cases.
Table 2: clinical presentation and examination of the studied group

<table>
<thead>
<tr>
<th>Clinical pictures</th>
<th>Studied group N=100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>nasal obstruction</td>
<td>100</td>
</tr>
<tr>
<td>bilateral</td>
<td>69</td>
</tr>
<tr>
<td>unilateral</td>
<td>31</td>
</tr>
<tr>
<td>Rhinorræ</td>
<td>90</td>
</tr>
<tr>
<td>bilateral</td>
<td>69</td>
</tr>
<tr>
<td>unilateral</td>
<td>21</td>
</tr>
<tr>
<td>itching</td>
<td>71</td>
</tr>
<tr>
<td>smell disorder</td>
<td>63</td>
</tr>
<tr>
<td>epistaxis</td>
<td>20</td>
</tr>
<tr>
<td>headache</td>
<td>82</td>
</tr>
<tr>
<td>bilateral</td>
<td>63</td>
</tr>
<tr>
<td>unilateral</td>
<td>19</td>
</tr>
<tr>
<td>Mouth breathing</td>
<td>24</td>
</tr>
<tr>
<td>Change of voice</td>
<td>4</td>
</tr>
<tr>
<td>proptosis</td>
<td>4</td>
</tr>
<tr>
<td>left side</td>
<td>2</td>
</tr>
<tr>
<td>rt side</td>
<td>2</td>
</tr>
<tr>
<td>EXamination</td>
<td>unilateral nasal polyp</td>
</tr>
<tr>
<td></td>
<td>bilateral nasal polyp</td>
</tr>
</tbody>
</table>

As regard the clinical diagnosis of the studied group, 84 cases were non-neoplastic (35 fungal, 45 non-specific and 4 antrochoanal), 13 were benign neoplastic (5 angiofibroma, 7 inverted papilloma and 1 haemangioma) and 3 cases were malignant. (table 3).

Table 3: Clinical diagnosis of the studied group.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Studied group N=100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>fungal nasal polyp</td>
<td>35</td>
</tr>
<tr>
<td>non specific</td>
<td>45</td>
</tr>
<tr>
<td>antrochoanal</td>
<td>4</td>
</tr>
<tr>
<td>benign tumour</td>
<td>13</td>
</tr>
<tr>
<td>angiofibroma</td>
<td>5</td>
</tr>
<tr>
<td>hemangioma</td>
<td>1</td>
</tr>
<tr>
<td>inverted papilloma</td>
<td>7</td>
</tr>
<tr>
<td>malignant tumour</td>
<td>3</td>
</tr>
</tbody>
</table>
The histopathological diagnosis of the studied group, were 84 cases (84%) non-neoplastic lesions, 44 cases allergic nasal polyps (eosinophil rich infiltrate), 24 cases was fungal polyp, 12 cases were non-specific inflammatory polyps, 4 cases were antrochoanal polyps, 12 cases were benign neoplastic lesions, 6 was inverted papilloma, 5 angiofibroma, only one case was hemangiomatous malformation. As regard malignant neoplastic lesion there was 4 cases.(table 4).

**Table 4: Histopathological findings among the cases (N=100).**

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Studied group N=100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>Allergic (n=68)</td>
<td>44</td>
</tr>
<tr>
<td>allergic nasal polyp (eosinophil rich</td>
<td>24</td>
</tr>
<tr>
<td>infiltrate)</td>
<td></td>
</tr>
<tr>
<td>fungal nasal polyp</td>
<td></td>
</tr>
<tr>
<td>Antrochonal Polyps</td>
<td>4</td>
</tr>
<tr>
<td>Non specific inflammatory nasal polyp</td>
<td>12</td>
</tr>
<tr>
<td>Benign tumor (n=12)</td>
<td>6</td>
</tr>
<tr>
<td>inverted papilloma</td>
<td>5</td>
</tr>
<tr>
<td>nasal angiofibroma</td>
<td>1</td>
</tr>
<tr>
<td>heamangiomatous malformation</td>
<td></td>
</tr>
<tr>
<td>Malignant tumor (n=4)</td>
<td>1</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>1</td>
</tr>
<tr>
<td>adenoid cystic carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>squamous cell carcinoma</td>
<td>2</td>
</tr>
</tbody>
</table>

Comparison of Clinically diagnosed cases with Histopathological diagnosis in Non-neoplastic cases (N=84), in 35 cases diagnosed clinically as fungal polyp, histopathological diagnosis was consistent with fungal polyp in 24 cases (68.6%) with significant p value (p<0.0001), 11 cases (31.4%) were diagnosed as allergic nasal polyp (eosinophil rich infiltrate) with non significant p value (p=0.063). in 45 cases diagnosed clinically as non-specific nasal polyps, histopathological diagnosis was consistent with non-specific inflammatory nasal polyps in 12 cases (26.7%) with significant p value (p<0.0001), 33 cases (73.3%) was diagnosed histopathologically as allergic nasal polyp (eosinophil rich infiltrate) with significant p value (p<0.0001). in 4 cases diagnosed clinically as antrochoanal polyps, histopathological diagnosis was consistent with antrochoanal polyp in 4 cases (100%) with significant p value (p<0.0001). (table 5).
Table (5): Comparison of Clinically diagnosed cases with Histopathological diagnosis in Non-neoplastic cases (N=84).

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Histopathological diagnosis</th>
<th>No.of patients</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>fungal polyp (N=35)</td>
<td>Consistent with fungal polyp.</td>
<td>24</td>
<td>68.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-specific nasal polyp (N=45)</td>
<td>Allergic(Eosinophil rich infiltrate)</td>
<td>11</td>
<td>31.4%</td>
<td>0.063</td>
</tr>
<tr>
<td>Antrochoanal polyps (N=4)</td>
<td>Consistent with Non-specific inflammatory</td>
<td>12</td>
<td>26.7%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Allergic(Eosinophil rich infiltrate)</td>
<td>33</td>
<td>73.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Non-specific inflammatory</td>
<td>4</td>
<td>100%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Comparison of Clinically diagnosed cases with histopathological diagnosis in neoplastic cases.(N=16), in 7 cases diagnosed clinically as inverted papilloma, histopathological diagnosis was consistent with inverted papilloma in 6 cases (85.7%) with significant p value(p<0.0001), 1 case(14.3%) diagnosed histopathologically as adenoid cystic carcinoma with non significant p value(p=0129). in 5 cases diagnosed clinically as angiofibroma, histopathological diagnosis was consistent with angiofibroma in 5 cases (100%) with significant p value (p<0.0001),in one case diagnosed clinically as heamangima, histopathological diagnosis was consistent with heamangima in also one case (100%) with significant p value (p<0.0001).in 3 cases diagnosed clinically as malignant lesion of nose and paranasal sinuses, histopathological diagnosis was consistent with malignant lesion in also 3 cases with significant p value (p<0.0001). (table 6).

Table (6): Comparison of Clinically diagnosed cases with Histopathological diagnosis in neoplastic cases (N=16).

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Histopathological diagnosis</th>
<th>No.of patients</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverted Papilloma (N=7)</td>
<td>Consistent with inverted papilloma.</td>
<td>6</td>
<td>85.7%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Adenoid cystic carcinoma</td>
<td>1</td>
<td>14.3%</td>
<td>0.129</td>
</tr>
<tr>
<td>Angiofibroma (N=5)</td>
<td>Consistent with Angiofibroma</td>
<td>5</td>
<td>100%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Haemangima (N=1)</td>
<td>Consistent with</td>
<td>1</td>
<td>100%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Malignancy of Nose &amp;PNS (N=3)</td>
<td>Hemangioma</td>
<td>3</td>
<td>100%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Consistent with malignancy</td>
<td>1</td>
<td>33.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Malignant melanoma</td>
<td>2</td>
<td>66.7%</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Nasal polyposis is a relatively common condition found in 1-4% of the general population (3). Our 100 patients of non-neoplastic and neoplastic lesions of the nose presenting as nasal polyps were studied in relation to the age-sex distribution, presenting symptoms, nasal endoscopy, radiological and histopathological findings. Mean age was 38.5 ± 8 years and more common in male than females. This was found to be similar to the study done by Larsen et al (4) whom study was conducted on 252 patients, they observed NP was more common in patients who were 40–60 years and more common in males than females (1.68 male and 0.82 female).

In another study done by Bakari et al (1) whom study was conducted on 76 patients, they found female preponderance (M:F ratio of 1:1.2). In our study the most frequent symptom was nasal obstruction (100%) and rhinorrhea (90%), followed by 71% with itching, 63% with smell disorders, 20% with epistaxis, 82% with headache and 4% with proptosis, 24% with mouth breathing and 4% with change of voice. Thahim et al (5) also found nasal obstruction in their 100% of cases, while nasal discharge was present in 90% of their patients. Bakari et al (1) whom study was conducted on 76 patients with sinonasal polyps, the main presenting symptoms were nasal obstruction (97.4%), rhinorrhea (94.7%). Out of 100 cases studied, 84 (84%) were non-neoplastic and 16 (16%) were neoplastic. Among the non-neoplastic conditions, allergic nasal polyps was the most common. Among the neoplastic lesions, 12 (12%) were benign and 4 (4%) were malignant. Among the benign neoplastic lesions, inverted papilloma was the most common, so non-neoplastic lesions was more common than neoplastic ones. This finding was similar to the study done by Lathi et al (6) whom study was conducted on 112 patients, they found that non-neoplastic was in 80 cases (71.4%) of the study subjects and neoplastic in 32 (28.6%) patients. In our study inverted papilloma was more common then angiofibroma then heamangioma.

Our results also coincided with the results of Sharma et al (7) whom study was done on 50 cases with sinonasal polyps, they found that non neoplastic lesions was more common (86%) of cases. Among benign neoplastic sinonasal masses, inverted papilloma was more common, it was found in 4% of cases then nasopharyngeal angiofibroma in 2% cases. Malignancy was found in 3 cases (6%).

Also the results coincided with a study done by Rawat et al (8), they found that found 68.56% of histopathological diagnosis as non neoplastic, 22.72% as benign and 8.71% as malignant.

As regards Comparison of Clinically diagnosed cases with Histopathological diagnosis in Non-neoplastic cases (N=84), in 35 cases diagnosed clinically as fungal polyp, histopathological diagnosis was consistent with fungal polyp in 24 cases (68.6%) with significant p value (p<0.0001), 11 cases (31.4%) were diagnosed as allergic nasal polyp (eosinophil rich infiltrate) with non significant p value (p=0.063). In 45 cases diagnosed clinically as non-specific nasal polyps, histopathological diagnosis was consistent with non-specific inflammatory nasal polyps in 12 cases (26.7%) with significant p value (p<0.0001), 33 cases (73.3%) was diagnosed histopathologically as allergic nasal polyp (eosinophil rich infiltrate) with significant p value (p<0.0001), in 4 cases diagnosed clinically as antrochoanal polyps, histopathological diagnosis was consistent with antrochoanal polyp in 4 cases (100%) with significant value (p<0.0001).

As regards comparison of Clinically diagnosed cases with histopathological diagnosis in neoplastic cases. (N=16), in 7 cases diagnosed clinically as inverted papilloma, histopathological diagnosis was consistent with inverted papilloma in 6 cases (85.7%) with significant p value (p<0.0001), 1 case (14.3%) diagnosed histopathologically as adenoid cystic carcinoma. In 5 cases diagnosed clinically as angiofibroma, histopathological diagnosis was consistent with angiofibroma in 5 cases (100%). in one case diagnosed clinically as hemangioma, histopathological diagnosis was consistent with hemangioma in also one case, in 3 cases diagnosed clinically as malignant lesion of nose and paranasal sinuses, histopathological diagnosis was consistent with malignant lesion in also 3 cases.

These results matched with results of a study done by Vaishali et al (9) histopathological diagnosis in Non specific polyps was consistent with clinical diagnosis in 12 (80%) cases while inconsistent in 3 (20%). In Allergic fungal polyps histopathological diagnosis was consistent in 7 (58.3%) and inconsistent in 3 (41.7%).
In diffuse polyposis with chronic rhinosinusitis 5(62. %%) and 3(43. 5%) were found consistent and inconsistent respectively with the histopathological diagnosis. All the clinically diagnosed cases of antrochoanal showed consistent reports with the histopathological diagnosis.

Comparison of Clinically diagnosed cases with Histopathological diagnosis in Neoplastic cases (n=6) showed only a single case clinically diagnosed of having malignancy of nose and PNS, histopathologically proved as cementoosiifying fibroma of the nose and PNS. Rest all the diagnostic consistency were the same as the clinical diagnosis. Further, it was analyzed that in Non-neoplastic cases(n=44) the Histopathological consisteny was in 33(75%) and inconsistency in 11(25%)This difference was statistically not significant.

The histopathological diagnosis correlated with clinical diagnosis in the present study in 55 cases(55%), in study done by Vaishali et al(9). the histopathological diagnosis correlated with clinical diagnosis in the present study in 38(76%) patients. In the study by Kale et al and Diamantopoulos II et al(10) the clinico – histopathological correlation was in 99. 7% and 98. 8% respectively. In our study the relatively lower clinico-pathological correlation, when compared to the other studies can be attributable to the lesser number of cases included in the study.

Conclusion

Non-neoplastic lesions was more common than neoplastic lesions, they are also more common in young ages and also are bilateral but neoplastic lesions are more common in the elderly and mostly unilateral. Sinonasal polyps are more common in males than females. because a lesion in the nasal cavity clinically presented as nasal polyp can either be neoplastic or non neoplastic and a significant lesions can be missed on either clinical or radiological evaluation ,so a thorough histopathological evaluation should be done in all cases of nasal polypoidal lesions for accurate diagnosis and management.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Given by the ethics committee of faculty of medicine ,Al-Azhar university.
A Study of Thyroid Function test and Lipid Profile in Pregnancy

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1Associate Professor, 2Tutor, Department of Biochemistry, 3Associate professor, Department of Forensic Medicine, 4Professor & Head, Department of Biochemistry, Mysore Medical College and Research Institute, Mysore

Abstract

Pregnancy is known to create profound changes in the body. It causes hormonal changes in the body which may lead to changes in lipid profile. TSH levels are usually suppressed due to very high levels of Human chorionic gonadotropin (hCG) during the first half of the pregnancy. Thyroid dysfunction may result in various changes in triglycerides, phospholipids, cholesterol and other lipoproteins. Dyslipidemia, a consequence of thyroid dysfunction, generally increases the risk for cardiovascular disease.

The study was conducted in the Department of Biochemistry, MMC&RI, Mysore. 100 women with a singleton pregnancy irrespective of parity and graida were enrolled and cases like chronic hypertension, Diabetes mellitus, Renal Disorders and Thyroid Disorders, Obstetric and Foetal Complications (Hydrops foetalis, congenital foetal anomalies) were excluded from the study. Blood was analysed for thyroid and lipid profile.

Conclusion: T3, T4 and TSH levels are within normal range during pregnancy. There is a positive correlation between TSH levels and cholesterol. This suggest that regular TSH and cholesterol estimation is very important during pregnancy to reduce the maternal and fetal complications.

Key words: cholesterol, dyslipidemia, HDL, LDL, lipid profile, VLDL and thyroid function test.

Introduction

Pregnancy is known to create profound changes in the body. It not only increases demand for metabolic fuels for the foetal growth and development but also causes hormonal changes in the body which may lead to changes in lipid profile1.

During early pregnancy, there is rise in serum estrogen and progesterone levels. And also, there is hyperinsulinemia leading to increased peripheral utilization of glucose, increased glycogen accumulation in the liver as well as increased storage of lipids and decreased lipid breakdown2.

The thyroid gland is an important endocrine gland in the human body because of its ability to produce the hormone triiodothyronine (T3) and tetraiodothyromine (T4). These hormones are important in regulating functions like growth, differentiation, cellular metabolism and general hormonal balance of the body, maintenance of metabolic activity and the development of skeletal and organ system. Over 99% thyroxine in the blood is bound to protiens like thyroxine binding globulin (TBG), albumin and prealbumin. Approximately 0.3% T4 is in the free unbound state in the blood. This free fraction is physiologically active due to its ability to enter target cells and influence calorigenesis and protein, lipid and carbohydrate metabolism. T4 also functions in the peripheral tissues as a prohormone by being further metabolized to another most active thyroid hormone, tri-iodothyromine (T3) and other inactive metabolites reverse T33.

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Mysore Medical College and Research Institute, Mysore. Email: mrarpitha@gmail.com
Tel. 9739651249
During pregnancy controlled hyper-stimulation of the ovaries results in a rise of serum TBG, T4 and T3 concentrations while there was a fall in serum free T4 concentration and a small rise in serum TSH concentrations (usually within the normal range). Also, TSH levels are usually suppressed due to very high levels of Human chorionic gonadotropin (hCG) during the first half of the pregnancy, especially the first trimester. Thyroid dysfunction without any primary thyroid disease may result in various qualitative or quantitative changes of triglycerides, phospholipids, cholesterol and other lipoproteins. Dyslipidemia, a consequence of thyroid dysfunction, generally increases the risk for cardiovascular disease.

Aim: The present study was undertaken to find out whether there is any significant variation in the thyroid function test and lipid profile during pregnancy and to establish a relation between TSH & lipid profile.

Materials & Method

The study was conducted in the Department of Biochemistry, MMC&RI, Mysore. This study was undertaken after obtaining the Institutional ethical clearance. The study was conducted for a period of 5 months. A total of 100 pregnant women were enrolled in the study after informed consent.

Inclusion criteria: All pregnant women with a singleton pregnancy who came to our hospital irrespective of parity and gravida.

Exclusion criteria: Pregnant women with diseases or complications like chronic hypertension, Diabetes mellitus, Renal Disorders and Thyroid Disorders, Obstetric and Foetal Complications (Hydrops foetalis, congenital foetal anomalies).

Collection of Blood Sample: 3ml of fasting blood was collected under aseptic precautions from the cubital vein. Serum was separated after centrifugation and analysed. T3, T4 and TSH were analysed by ECLIA method. The serum total cholesterol by enzymatic CHOD-PAP method, serum triglycerides by enzymatic GPO-PAP method, serum high density lipoprotein (HDL) cholesterol by Direct enzymatic method, very low density lipoprotein (VLDL) by calculation method (triglycerides/5) and low density lipoprotein (LDL) cholesterol was calculated by Fredrickson-Friedwald formula \{total cholesterol - HDL cholesterol - (triglycerides/5)\}.

Statistical Method: The data was entered in excel format and analysed using epi-info software. Descriptive statistics like frequency and percentage were calculated. Association between qualitative variables was tested by chi-square test. P value less than or equal to 0.05 was considered as significant. Correlation was used for quantitative variables. The values between 0 to +1 was considered as positive correlation and 0 to -1 as negative correlation.

Results & Discussion

Table 1: Thyroid function test of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>171.72 ng/dl</td>
<td>4.04 ng/dl</td>
</tr>
<tr>
<td>T4</td>
<td>10.73 µg/dl</td>
<td>48.07 µg/dl</td>
</tr>
<tr>
<td>TSH</td>
<td>2.18 µIU/ml</td>
<td>2.16 µIU/ml</td>
</tr>
</tbody>
</table>

Table 2: Thyroid status of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 3: Lipid profile of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>223.32 mg/dl</td>
<td>58.87 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>198.38 mg/dl</td>
<td>76.46 mg/dl</td>
</tr>
<tr>
<td>HDL</td>
<td>60.9 mg/dl</td>
<td>11.89 mg/dl</td>
</tr>
<tr>
<td>LDL</td>
<td>106.8 mg/dl</td>
<td>26.47 mg/dl</td>
</tr>
<tr>
<td>VLDL</td>
<td>39.67 mg/dl</td>
<td>15.29 mg/dl</td>
</tr>
</tbody>
</table>

Table 4: Lipid status of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 5: Association between TFT profile and lipid profile of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal lipid profile</th>
<th>Hypercholesterolemia</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid (Normal TSH)</td>
<td>30</td>
<td>60</td>
<td>90</td>
<td>0.092</td>
</tr>
<tr>
<td>Hypothyroid (high TSH)</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hyperthyroid (low TSH)</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>70</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Correlation between TSH and cholesterol

<table>
<thead>
<tr>
<th>Mean TSH</th>
<th>Mean</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.18 µIU/ml</td>
<td>223.32 mg/dl (Total cholesterol)</td>
<td>+0.019</td>
</tr>
<tr>
<td></td>
<td>60.9 mg/dl (HDL)</td>
<td>+0.35</td>
</tr>
<tr>
<td></td>
<td>106.8 mg/dl (LDL)</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Pregnancy is a stress condition to the maternal thyroid gland, due to increase in thyroxin binding globulin, increased demand for iodine and thyroid stimulation by HCG. Thyroxin is an important hormone for fetal brain development, growth and lung maturation. Thus if maternal levels are not well maintained in pregnancy, fetus is at risk. Hence regular measurement of thyroid profile is important.

Table 1 & 2 shows Thyroid function test of study subjects. Out of 100 subjects, 90% were euthyroid, and 8% & 2% were hypothyroid & hyperthyroid.
respectively. The mean T3 level was 171.72 + 48.07ng/dl, mean T4 was 10.73 + 2.16µg/dl and mean TSH was 2.18 + 1.37 µIU/ml. Patwari M et al also showed similar results. According to Patwari M et al, the mean T3, T4 & TSH was 71-175ng/dl, 3.6-9.0µg/dl & 0.1-4.0 µIU/ml; 84-195 ng/dl, 4.0-8.9µg/dl & 0.4-5.0 µIU/ml; 97-182 ng/dl, 3.5-8.6µg/dl & 0.23-4.4 µIU/ml in 1st, 2nd and 3rd trimester respectively. When average values of all the 3 trimesters were considered the results were in agreement with present study.

Thyroid hormones play an important role in synthesis, mobilization and metabolism of lipids. Therefore, hypothyroidism is a major cause of secondary dyslipidemia. The risk of developing atherosclerosis is directly related to the plasma cholesterol. Increased level of cholesterol for prolonged period will favor deposits in subintimal regions of arteries. The oxidized LDL (ox-LDL) gets deposited in the walls of arteries, which are degraded by nonspecific uptake by macrophage. There is also smooth muscle cell proliferation and fibrosis. So, this adversely affects the pregnancy. In the present study we have carried out the lipid profile and found dyslipidemic features.

Table 3 & 4 shows lipid profile of subjects. Out of 100 subjects, 70% were Hypercholesterolemia. The mean total cholesterol was 223.32 + 58.87mg/dl, triglycerides was 198.38 + 76.46 mg/dl, HDL 60.9 + 11.89 mg/dl, LDL was 106.8 + 26.47mg/dl and VLDL was 39.67 + 15.29 mg/dl. There is a consensus regarding elevated levels of total cholesterol, triglycerides, LDL and VLDL between the present study and the study done by Pusukuru, R et al., on 200 pregnant women. Pusukuru, R et al. learnt that the average values of total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein in 2nd and 3rd trimester was 228.61mg/dl, 202.73 mg/dl, 46.09 mg/dl, 115.11 mg/dl & 32.24mg/dl respectively.

Table 5 shows association between TFT and Lipid profile. Out of 90 euthyroid subjects 60 were in Hypercholesterolemic status but it was statistically not significant (p > 0.05).

Table 6 shows correlation between TSH and cholesterol levels and we observed a positive correlation between total cholesterol and HDL. This indicates that the TSH and Cholesterol & HDL are positively associated. When TSH level increases, cholesterol levels also increases. Garduno-Garcia JJ et al. concluded that TSH is significantly associated with lipid profile in the euthyroid population and they showed TSH is positively correlated with TC, TG & LDL and negatively correlated with HDL.

Pregnancy is a phase of life, where the emotions are high not only to the pregnant lady but to the other family members. If there is any untoward incidence during pregnancy, then allegations of medical negligence are often cited as the reason. Hence, the study which establishes association between TFT and lipid profile to maternal and fetal complications are need of the hour. It is recommended that forensic experts consider regularly examining the thyroid gland histologically, particularly a cause of death there is no apparent anatomic cause of death in pregnant women.

Conclusion

T3, T4 and TSH levels are within normal range during pregnancy. There is a positive correlation between TSH levels and cholesterol. This suggest that regular TSH and cholesterol estimation is very important during pregnancy to reduce the maternal and fetal complications. The documentation in the form of tests of Thyroid function tests and Lipid profile will serve as a arsenal to the doctors in cases of alleged Medical Negligence. Lack of documentation can be an achille’s heel even though doctor would have taken all precautions.

Limitations: The sample size is less to establish proper correlation between thyroid status and dyslipidemia and its related complications. Hence, the study should be conducted on large sample size and these parameters should be considered in all trimesters and postpartum period as well.

Conflict of Interest: Nil

Source of Funding: Self-funding

Ethical Clearance: Taken from Mysore Medical College and Research Institute Ethical Committee

References


Caracteristics, Diagnosis, Management and Output of Persistent Pulmonary Hypertension of the Newborn at Dr. Soetomo Hospital

Mardiyan Aprianto¹, Mahrus A Rahman¹, Martono Tri Utomo¹

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Abstract

Background Persistent pulmonary hypertension of the new-born (PPHN) is a failure of lung circulation in new-born. However the data related the characteristics of clinical profile, diagnostic, management and the outcome was still limited.

Objective To investigated the characteristics, diagnostics, management and outcome from PPHN.

Methods Retrospective observational study. Neonates with PPHN from January 2015 to December 2019 were identified from medical record. After the data was excluded, the characteristics, diagnostic, management and the outcome was collected. The statistical analysis to known the frequency and the chi-square test used to analyse the association between the treatment and the outcomes ($P < 0.05$).

Results 37 medical records enrolled the study, with the characteristics; 62.2% babies was boy, 70.3% with term infant, 70.3% with normal birth weight, 24.3% with maternal history of eclampsia. 62.2% babies born by C-section, 21.9% with history of asphyxia and MAS. 78.4% with differences between pre- and post-ductal saturation. From echocardiography result, 13.5% diagnosed as mild PPHN, 54.1% as moderate PPHN and 32.4% severe PPHN. From the management consisted of 5.4% with O₂ nasal, 32.4% O₂ CPAP and 62.2% O₂ ventilator, 24.3% with sildenafil, 5.4% with combination sildenafil and illoprost, 5.4% with combination sildenafil, illoprost, and surfactant, 2.7% with combination sildenafil, illoprost, surfactant, and inotropic, 24.3% with combination sildenafil, illoprost, and inotropic, and 37.8% with combination sildenafil and inotropic. The outcomes was obtained 35.1% babies was died and 64.9% babies was cured, with oxygenation supplementation had significantly affecting the outcomes ($P=0.02$)

Conclusion The characteristics of PPHN was dominated by baby boy, term infant and good birth weight, history of asphyxia and MAS, maternal history of eclampsia, C-section delivery and the differences between pre- and post-ductal saturation. The diagnosis commonly with moderate PPHN. The management with O₂ ventilator and combination sildenafil-inotropic. The outcome mostly the babies was cured.

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Keywords: Persistent pulmonary hypertension of the new-born, Characteristic, Diagnostic, Management, Outcome

Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is a syndrome lung circulation failure to transition from fetal circulation to neonatal circulation.
Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1

The incident PPHN is quite rare, in worldwide ranging from 1-6 over 1000 of live births. Based on medical record from 2015 to 2016 In Dr. Soetomo Hospital were found 20 cases. This condition generally life-threatening with the prognosis tends to be poor, especially in developing countries\(^1,5,14,22\). Samudro and Mulyadi (2012) in their study explained, PPHN with the delivery history followed with low birth weight, presence of infection, and labor procedures with C-section, tends to have a poor prognosis. However, the data in Indonesia is still incomplete\(^20\).

In the world the mortality rate infants with PPHN is around 10% and will increases if followed by congenital anomaly such as diaphragmatic hernias. For the morbidity rate it was around 25% consist of developmental disorders, pulmonary hypertension and neurological disorders\(^1\).

Walsh-Sukys MC (2000), infant with PPHN have several clinical profile characteristics, commonly happened in baby boy and white race ethnic\(^23\). Another clinical profile that PPHN could presence in parenchymal lung disease in the newborns, include of meconal aspiration syndrome (MAS) and or respiratory distress syndrome (RDS). That is the reason why PPHN could occur in preterm or term infants \(^8,20\). In addition, characteristic of infant with history of intrauterine growth restriction (IUGR), hypoglycemia, polycythemia, or oligohydramnios are also suspected as the risk factors of PPHN \(^2,20\).

Diagnosing PPHN is not easy, sometimes it can underdiagnosed and considered a congenital heart disease (CHD), so the close initial evaluation is needed especially in infants with history born with hypoxia. Characteristic of maternal history also important, because mother with diabetes, asthma, hypertension, eclampsia, obesity and drug used during pregnancy was suspected as a risk factor, history of delivery, physical examination by measure the pre- and post-ductal oxygen saturation also important to know. As the gold standard to diagnosed PPHN, echocardiography still preferred. With echocardiography examination we can found the anatomical disorder and also to monitor the effectiveness of the therapy was given \(^9\).

After the diagnosed already established, the therapy for PPHN will be given based on the severity. The goal of the PPHN management are to overcoming circulatory failure and improving oxygenation needed. However, in Dr. Soetomo Hospital there were known several combinations therapy and the clear data about the right combination therapy in order to obtain the best outcome is still unknown. Management therapy at Dr. Soetomo Hospital currently includes many combination therapy consist of oxygenation supplementation, with surfactants, or vasorelaxants, or phosphodiesterase inhibitors, or illoprost inhalation and or inotropes administration\(^1,13,19,20,22\). Therefore, to obtain a good outcome babies with PPHN, the proper diagnosis and good management evaluation are needed to achieve optimal conditions \(^1,19\).

The aimed of this study to investigated the characteristics of clinical profile, diagnostics, management and outcome from PPHN.

**Material and Methods**

A retrospective observational study, with the neonates who diagnosed as PPHN that hospitalize in NICU room and the data was collected based on medical records. The data was collected from January 2015 to December 2019. The inclusion criteria for the medical record consist of the infants with aged since birth to 30 days old and already diagnosed as PPHN, confirmed by echocardiography, then got management therapy according to applicable guidelines. The statistical analysis was to calculate the frequency (by presentation) and the chi-square test used to analyse the association between the treatment and the outcomes \((P < 0.05)\).

**Operational Definition**

**Persistent pulmonary hypertension of new-born** defined as increased pulmonary vascular resistance in neonates characterized respiratory distress, followed by shunting of blood circulation from the right to the left heart chamber with clinical severe hypoxemia \(^16\), and already confirmed by echocardiography \(^18\).

The characteristics PPHN are clinical profile included sex and the risk factors during gestational age, infant birth weight, history of delivery, maternal history (eclampsia, premature rupture of membranes (PRoM), oligohydramnios, bleeding during pregnancy, and history of drugs consumption), history of labor procedure (spontaneous or C-section), history of natal
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(RDS, asphyxia, MAS, congenital heart disease, HIE and diaphragmatic hernia), and complications during hospitalization.

The diagnosis of PPHN in infants based on clinical symptom with respiratory distress followed by hypoxia that occurs in the first 6-12 hours after birth and the history of risk factors PPHN, from physical examination with differences more 10% between pre- and post-ductal saturation, confirmed with echocardiography. From echocardiography result of shunting from right to left through patent ductus arteriosus (PDA) and or patent foramen ovale (PFO), increased right ventricular pressure and pulmonary artery pressure with tricuspid regurgitation (TR), or found right ventricular dysfunction. The severity of PPHN are divided into mild PPHN (echo result mild TR), moderate PPHN (moderate TR) and severe PPHN (severe TR)

The management therapy of PPHN included oxygenation (administration through nasal canul / CPAP / mechanical ventilator) and drug administration (sildenafil; sildenafil with illoprost; sildenafil, illoprost, and surfactant; sildenafil, illoprost, surfactant and inotropic; sildenafil, illoprost and inotropic; and sildenafil with inotropic). The purpose of management to increase oxygen supply, reduce oxygen demand, facilitate adequate gas exchange in the lungs, reduce pulmonary vascular pressure by increasing vasodilation of pulmonary blood vessels, improving mixing of flow in the cardiac atrium and through PDA and improving metabolic disorders.

The outcome of PPHN after evaluation and treatment defined as recovery (without sequelae or with sequelae) or died.

Results

In this study, were found 56 medical records infants with PPHN, but only 37 medical record still remained. The characteristics in this study based on sex 62.2% dominated with baby boy with a boy to girl ratio was 1.6 : 1. Based on the maternal history in this study consisted of 24.3% with eclampsia, 18.9% with PRoM, 13.5% with oligohydramnios, 5.4% with bleeding during pregnancy, 70.3% with appropriate gestational age consisting of term-infant 70.3% and preterm infants 29.7%. Based on the history of childbirth 62.2% babies were born with C-section and 37.8% were born spontaneously, and according to birth weight 70.3% with normal birth weight, 24.3% infants with low birth weight and infants with very low birth weight (5.4%) (Table I).

The characteristic of perinatal history, there were 21.6% infants with asphyxia neonatorum, 21.6% with MAS, 10.8% with RDS, 5.4% with hypoxic ischemic encephalopathy (HIE), 2.4% with diaphragmatic hernia and 2.7% with congenital heart disease. The complications during hospitalization consist of pneumonia (45.9%), and sepsis (43.2%), with length of stay about 10 days.

Table II showed the diagnostic from babies with PPHN based on differences between pre- and post-ductal saturation, confirmed with echocardiography as the gold standard diagnostic. From this study was found 78.4% with the differences between pre- and post-ductal saturation and from echocardiography was confirmed 13.5% with mild PPHN, 54.1% with moderate PPHN dan 32.4% with severe PPHN.
Table I. The characteristics persistent pulmonary hypertension of the newborn

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Category</th>
<th>n (n=37)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Boy</td>
<td>23</td>
<td>62.2</td>
</tr>
<tr>
<td></td>
<td>Girl</td>
<td>14</td>
<td>37.8</td>
</tr>
<tr>
<td>Maternal history</td>
<td>Eclampsia</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>PRoM</td>
<td>7</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>Oligohydramnion</td>
<td>5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>Bleeding during pregnancy</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Gestasional age :</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Term infant</td>
<td>26</td>
<td>70.3</td>
</tr>
<tr>
<td></td>
<td>Preterm infant</td>
<td>11</td>
<td>29.7</td>
</tr>
<tr>
<td>Delivery history</td>
<td>C-Section</td>
<td>23</td>
<td>62.2</td>
</tr>
<tr>
<td></td>
<td>Spontaneous</td>
<td>14</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>Birth weight :</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal birth weight</td>
<td>26</td>
<td>70.3</td>
</tr>
<tr>
<td></td>
<td>Low birth weight</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>Very low birth weight</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Extremely low birth weight</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Perinatal history</td>
<td>Asphyxia</td>
<td>8</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>MAS</td>
<td>8</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>RDS</td>
<td>4</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>HIE</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Diafragmatic Hernia</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>CHD</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>Complication</td>
<td>Pneumonia</td>
<td>17</td>
<td>45.9</td>
</tr>
<tr>
<td></td>
<td>Sepsis</td>
<td>16</td>
<td>43.2</td>
</tr>
<tr>
<td>Length of stay (days)</td>
<td>Mean (min-max)</td>
<td>10 days (2-18 days)</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Diagnostic confirmation of PPHN

<table>
<thead>
<tr>
<th>Diagnostic result</th>
<th>Category</th>
<th>Total (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>The differences between pre- and post-ductal saturation</td>
<td>Yes</td>
<td>29</td>
<td>78.4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>8</td>
<td>21.6</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>Mild PPHN</td>
<td>5</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>Moderate PPHN</td>
<td>20</td>
<td>54.1</td>
</tr>
<tr>
<td></td>
<td>Serious PPHN</td>
<td>12</td>
<td>32.4</td>
</tr>
</tbody>
</table>


Table III showed the management for PPHN consist of oxygenation supplementation and drug combination therapy. In this study was found 5.4% was got O$_2$ nasal, 32.4% with O$_2$ CPAP and 62.2% with mechanical ventilator support. For drug combination therapy was found 24.3% was got sildenafil, 5.45 with combination of sildenafil and illoprost, 5.4% with combination of sildenafil, illoprost, and surfactant, 2.7% with combination of sildenafil, illoprost, surfactant, and inotropic, 24.3% with combination of sildenafil, illoprost, surfactant, and inotropic and 37.8% with combination of sildenafil and inotropic.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Total (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenation</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>O2 nasal</td>
<td>12</td>
<td>32.4</td>
</tr>
<tr>
<td>O2 CPAP</td>
<td>23</td>
<td>62.2</td>
</tr>
<tr>
<td>Mecanical ventilator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sildenafil</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td>Sildenafil + Illoprost</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Sildenafil + Illoprost + surfactant</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Sildenafil + Illoprost + surfactant + Inotropic</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>Sildenafil + Illoprost + Inotropic</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td>Sildenafil + Inotropic</td>
<td>14</td>
<td>37.8</td>
</tr>
</tbody>
</table>

Table IV showed the outcome of PPHN based on the diagnostic from echocardiography. In this study was found 64.9% PPHN cured which are 13.5% with mild PPHN, 45.9% with moderate PPHN and 5.4% with severe PPHN. 35.1% PPHN was died, which are 8.1% with moderate PPHN and 27% with severe PPHN.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mild PPHN n (%)</th>
<th>Moderate PPHN n (%)</th>
<th>Severe PPHN n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured</td>
<td>5 (13.5)</td>
<td>17 (45.9)</td>
<td>2 (5.4)</td>
<td>24 (64.9)</td>
</tr>
<tr>
<td>Died</td>
<td>0 (0.0)</td>
<td>3 (8.1)</td>
<td>10 (27.0)</td>
<td>13 (35.1)</td>
</tr>
</tbody>
</table>

Table V to show association between the outcome to the management and to the complication. In this study was found oxygenation support have significant association with the outcome of PPHN ($P < 0.05$).
### Tabel V. Bivariant analysis of PPHN

<table>
<thead>
<tr>
<th>Management therapy</th>
<th>Cured n (%)</th>
<th>Died n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>· Sildenafil</td>
<td>9 (24.3)</td>
<td>0 (0.0)</td>
<td>0.052*</td>
</tr>
<tr>
<td>· Sildenafil + Illoprost</td>
<td>2 (5.4)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>· Sildenafil + Illoprost + surfactant</td>
<td>1 (2.7)</td>
<td>1 (2.7)</td>
<td></td>
</tr>
<tr>
<td>· Sildenafil + Illoprost + surfactant + Inotropic</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>· Sildenafil + Inotropic</td>
<td>6 (16.2)</td>
<td>3 (8.1)</td>
<td></td>
</tr>
<tr>
<td>· Sildenafil + Inotropic</td>
<td>6 (16.2)</td>
<td>8 (21.6)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oxygenation :</th>
<th>Cured n (%)</th>
<th>Died n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 nasal</td>
<td>2 (5.4)</td>
<td>0 (0.0)</td>
<td>0.02*</td>
</tr>
<tr>
<td>O2 CPAP</td>
<td>12 (32.4)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilator</td>
<td>10 (27.0)</td>
<td>13 (35.1)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complication</th>
<th>Cured n (%)</th>
<th>Died n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>· Sepsis</td>
<td>11 (29.7)</td>
<td>5 (13.5)</td>
<td>0.189*</td>
</tr>
<tr>
<td>· Pneumonia</td>
<td>9 (24.3)</td>
<td>8 (21.6)</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test

### Discussion

Research on persistent pulmonary hypertension of the newborn (PPHN) has been widely carried out, but the results obtained are still unsatisfactory. The occurrence of pulmonary vascular disorders in infants is known to be the cause of PPHN. Based on the pathophysiology, the disorder is divided into three, namely under-development of pulmonary vascularization during the intrauterine period, the presence of mal-development (abnormalities of the vascular structure), and or the presence of maladaptation (generally due to vascular spasm triggered by perinatal hypoxia). Currently, several characteristics was thought to be the predisposing factors for PPHN have been identified. In this study, the characteristics of PPHN based on gender 62.2% was boy and 37.8% girls with a ratio of 1.6: 1. Choudhary’s (2015) in his study, baby boy were also dominated 65% from all sample, as well as in Harish and Kamalarathnam’s (2018), where the ratio of between boy and girl was 1.5: 1.

The characteristic of PPHN based on maternal history, in this study was found 24.3% with eclampsia, 18.9% with history of premature rupture of membranes (PRoM), 13.5% oligohydramnion and 5.4% with placenta previa. Eclampsia and bleeding during pregnancy caused intrauterine hypoxia due to reduced oxygen supply that was distributed to the foetus. It will exacerbate the pulmonary transition process as a risk of developing PPHN that occurs when the baby was born. Bleeding during pregnancy will also cause foetal anemia, it will lead into the abnormalities pulmonary vascularization development (known as mal-development). The mother’s condition followed by PRoM can cause pulmonary hypoplasia in infants as the results from oligohydramnios that occur during PRoM progress. It will lead to the abnormalities in parenchyma and the development of the pulmonary vascular. It also will decrease the number of blood volume and trigger vasoconstriction of pulmonary blood vessels. This process is known as underdevelopment that can caused PPHN. For management this condition required generally prolonged cardio-respiratory support because sometime not responding to vasodilators administration. Gestational age also known as the characteristic of PPHN. In this study, PPHN 70.3% occurred at term infant compared to babies born preterm. Research by
Roofthooft (2011) found PPHN occurred mostly at term infant than preterm infant. Harish S and Kamalarathnam C (2018), also showed 82.3% of PPHN occurred in term infant. The characteristics of the gestational age babies with PPHN in each health care facility can be different. The preterm babies, generally all the organs in an under-development condition, especially the lung. The under-development of the lungs will trigger hypoxia that in long term could be the risk factor of chronic lung disease as the secondary cause of PPHN. It will be worsened if followed by high pulmonary vascular resistance (PVR) due to failure of the post-natal transition. In term infants the mechanism of PPHN is quite different, it due to maladaptation of the pulmonary parenchyma (eg, in MAS or RDS conditions) that also caused high PVR during the post natal transition.

The history of labor, included the procedure of delivery and the birth weight also known as the characteristic of PPHN. In this study 62.2% PPHN was found in C-section compared to spontaneous delivery was only 37.8%. Choudhary et al (2015), 57.89% PPHN found in babies who’s born by C-section, as well as in research of Harish S and Kamalarathnam C (2018), the incidence of PPHN in babies with history of C-section about 68.9% PPHN in infants who’s born by C-section is caused by the interference of endogenous catecholamine secretion. Endogenous catecholamine in infants are important during the transition process. Disruption of it caused delayed the transition process, and also trigger exchange disorders in the lungs due to the lack of stimulation of the lung and significantly triggers immaturity of the lungs. The differentiated with spontaneous delivery can provide mechanical stimulation in the form of manual compression on the chest wall and stimulates the baby to breathe, also helps to expelling amniotic fluid in the respiratory track. However, this does not conclude that C-section is prohibited, because there are several indications that require the procedure to be performed.

In this study, PPHN occurred in 70.3% babies with normal birth weight, then low birth weight. Harish S and Kamalarathnam C (2018) found, 68.5% incidence PPHN happened in babies with moderate birth weight compared to babies with low birth weight. PPHN in sufficient birth weight occurs due to secondary factors that cause maladaptation of the lung (babies born with MAS or transient tachypnea of newborn). But it didn’t mean PPHN couldn’t occurred in low birth weight infant, it just happen due to the under-development of the lungs tissues and vascularisation. The PPHN in low birth weight that identical to intrauterine growth retardation (IUGR), was caused by under-development mechanism of the lung. So, the infants with sufficient birth weight or less, each of them has the possibility to suffer PPHN.

The perinatal history related to PPHN, in this study was found that neonatal asphyxia and MAS had the same incidence of PPHN (21.6%), followed by RDS, hypoxic ischemic encephalopathy (HIE), diaphragmatic hernia and congenital heart disease. Other characteristics are complications that occur during the hospitalization. The complications in our study consist of pneumonia (45.9%) and sepsis (43.2%). Sharma (2011), PPHN most often occurs in infants with a history of asphyxia and MAS because clinical hypoxia which can trigger pulmonary vascular spasm. In the normal physiological condition, the air exchange process was done by placenta would be replaced by the lung, after the baby born, followed by increased of pulmonary oxygenation through complex biochemical mechanisms and processes. This will facilitate the transition process from the new born, but if it got disruption it will lead to failure of pulmonary development as well as vascular dysfunction. The mechanism known as maladaptation.

Roofthooft et al (2011) in their research, as addition infections (pneumonia, and or sepsis), congenital disorders such as diaphragmatic hernia and congenital heart disease can cause PPHN. These factors caused maladaptation of pulmonary development. In addition, there was other mechanisms that could trigger PPHN due to abnormalities in the development of pulmonary vascularization (maldevelopment) which are generally idiopathic, for example in chronic foetal hypoxia, foetal anemia, or with congenital heart disease (PDA). The third mechanism that can trigger PPHN is the underdevelopment mechanism, for example in the condition of lung hypoplasia that occurs in infants with diaphragmatic hernias and infants with infections. Infection is a risk factor of sepsis, as well as pneumonia. Infection will cause damage of lung parenchyma through surfactant inactivation mechanisms, release of pro-inflammatory mediators, and other chemical...
reactions that increase the secretion of vasoconstrictors such as endothelin and thromboxane. If the condition is exacerbated by hypoxemia that occurs since birth, it will aggravate vasoconstriction of the pulmonary vascular. On the other hand, if the sepsis not resolved, will decrease the systemic vascular resistance (SVR) with clinical hypotension. If the condition followed by an increase in PVR, it will be trigger shutting via from the right to the left heart and made the PPHN more severe. Konduri and Kim (2009) explained that sepsis in PPHN was caused by infection with Group B Streptococcus and gram-negative organisms that acquired during hospitalization. The bacterial endotoxin released in the bloodstream caused pulmonary hypertension, these mechanisms made thromboxane, endothelin and cytokines release and the over-activation of nitric oxide. It also cause worst effect such as multi-organ failure including myocardial dysfunction.

Diagnosis of PPHN is not easy, from history taking of maternal and childbirth followed by a physical examination, supporting the diagnosis to detect PVR which is the characteristic of PPHN, as well as to find the condition of blood flow and myocardial function. Supportive non-invasive examinations are recommended PPHN. The recommendations suggest the pre- and post-ductal ratio of oxygen saturation (\(\text{SpO}_2\)) and echocardiography as the gold standard. From saturation that showed 5-10% difference between the right upper limb (pre-ductal) and the lower left (post-ductal) limb indicated the PPHN, but the disadvantages of this examination is unable to confirm heart structural abnormalities. This saturation measurement was carried out during the first 6-24 hours of life, after clinical signs of hypoxia were found. This examination is very helpful as the initial screening of PPHN, especially in limited health facilities before confirmed by echocardiography. In this study, it was found that 78.4% of the newborn with differences of pre- and post-ductal saturation and 21.6% were not have it. The difference in pre- and post-ductal saturation in PPHN infants occurs due to episodic changes in circulating pulmonary blood flow and shunting from the right to the left heart chamber. If the changes of pulmonary circulation occur more intense, it mean the condition developed severely.

Echocardiography still recommended by 95% of respondents as the gold standard to diagnosed PPHN. This examination is easy to use and can be done bedside if the patient’s condition was unstable. This examination is also used to assess progressivity or how the response of therapy that has been given. Echocardiography used to determine the cardiac structural abnormalities and to rule out the differential diagnosis as cyanotic CHD, because from the physical examination, both of it can followed by systolic murmurs due to tricuspid regurgitation or continuous murmurs from PDA. From echocardiography we can diagnosed PPHN if there was right ventricular hypertrophy, deviation of the interventricular septum to the left side, tricuspid regurgitation (TR), and right to left bidirectional shunting via PFO and PDA. In this study was found, from echocardiography 13.5% patient with mild PPHN, 41.1% with moderate PPHN, and 32.4% with severe PPHN.

Oxygenation therapy in this study was found 5.4% infants received nasal \(\text{O}_2\) therapy, 32.4% infants received \(\text{O}_2\) CPAP and 62.2% with mecanical ventilator. The purpose of oxygenation management is to fix hypoxemia and improve the blood pressure, which it will effect reduction of the heart shunting from the defect. If the condition followed by persistent hypoxia, with clinical possibility of PPHN, then the first step is to provide oxygenation until the diagnosis of PPHN has been confirmed. Oxygen supplementation acts as a potent vasodilator and to assist post-natal physiological adaptation processes based on oxygen needs. However, during the oxygenation therapy it important to monitoring and choose the right way to give the oxygenation according to the severity so the oxidative stress could be prevented. Because if oxidative stress occurs, it will form reactive oxygen species which cause the opposite effect (vascular vasoconstriction).

The management of PPHN, in this study consisted 24.3% received a combination of sildenafil and illoprost, 5.4% received a combination of sildenafil, illoprost, and surfactant, 2.7% received a combination of sildenafil, illoprost, surfactant and inotropic, 24.3% received a combination of sildenafil, illopros, and inotropic and 37.8% infants received sildenafil and inotropic combination. Indication of therapy based on the clinical condition of patient and
the availability of the therapy in dealing with PPHN in each hospital. In this study, sildenafil commonly used in all infants especially with mild to severe PPHN. Sildenafil known as a phosphodiesterase inhibitor type 5 (PDE5) which selectively to reduce PVR and increase cyclic guanosinemonophosphate (cGMP), it cause a vasodilating effect the vascular. If the administration of sildenafil was combine with oxygen supplementation, it will give improvement and oxygenation needs 25.

Surfactant in PPHN especially indicated for infants with a history of MAS. Physiologically 5% -24% of the newborn can experience meconium staining of amniotic fluid (MSAF), 5% of this MSAF could develops into MAS and caused hypoxemia due to obstruction of the airway and surfactant inactivation 14. Surfactant administration in the newborn will expected to stimulate the release of meconium from the airways, improve lung oxygenation and reduce shunting flow from the right to the left heart 14, 19.

The other recommended therapy in management of PPHN is the administration of vasorelaxants, which namely as illoprost. Vasorelaxants act by inhibiting the transport of calcium ions in lung and systemic vascular smooth muscle cells. The effect is to reduce vasoconstriction of blood vessels, but during the administration it needs to be monitored closely because can make systemic side effect in the form of hypotension. Illoprost can be given by inhalation especially in PPHN with severe hypoxia suspected caused by unclear cyanotic heart disease. When it got combined with sildenafil will give better results 1, 19. In PPHN with clinical symptom persistent hypoxemic and hypotension. We could suspected increased of shunting right to left shunt was occur. Hypotension caused by systemic vascular resistance (SVR) was decrease and followed with increased shunting form. Inotropic agents is one of the methods to reduce the shunting, but depends to considerably in each health care facility, because there is no clear evidence which group is the best. Nakwan (2016), said the inotropes dopamine was choose as the first-line therapies, dobutamine and epinephrine will be selected as the second and third line therapies 16.

The outcome in this study was found that 24 patient was recovered, which 37.8% infants cured without sequelae, and 13 patient was died. The outcome of the patient who died especially in limited health care facilities tended to be high. Harish and Kamalarathnam (2018), in their study in India, said that the mortality rate of PPHN could reached 42.3% even though these babies had received sildenafil therapy and mechanical ventilators. Harish and Kamalarathnam (2018) suspect that the cause of death is due to inappropriate therapy. Ideally, oxygenation therapy should be accompanied by iNO (inhaled nitric oxide) or using the HFO (high frequency oscillation) or ECMO (extracorporeal membrane oxygenation) method. The obstacle is, the modality of the therapy is expensive and requires inexpensive technicalities 4, 5, 7. iNO known has the fast effect and selective working site at pulmonary vascular (Luecke and Mcpherson., 2017). This mechanism caused vasodilator by involves the production of cyclic guanosine monophosphate (cGMP) in the muscularis lining of blood vessels, then it will activates cGMP kinase and causes calcium pump inhibitors in blood vessels. Decrease of calcium ions that enter the smooth muscle cells will trigger relaxation and vasodilation of pulmonary blood vessels. If the therapy combined with sildenafil, the effect of suppression of cGMP regulation will be prevented and the vasodilation can have a long half-time 26. The other mechanism of iNO is to preventing anaerobic metabolism during hypoxia, by changes the hemoglobin binding that effect oxygenation supply improved 27. The ECMO and HFO ventilation also can provide improvement for the lung oxygenation up to 53%, compared to regular mechanical ventilation in PPHN infants, with recovery rates from 81-82% 11, 27. The combination of HFO and iNO also give the advantage, because with low pressure it can quickly corrected arterial oxygenation saturation 1, 3.

In this study, the oxygenation supplementation had a significant effect to the outcomes. Oxygenation is the first line therapy which acts as a major stimulant of the nitric oxide and prostacyclin pathways of the pulmonary vasodilator cascade. The advantage from oxygenation to PPHN are fast action and selective onset as a vasodilator for pulmonary vascularization and provides negative feedback to vasoconstriction stimulation in the central nervous system 27. This oxygen supplementation can be adjusted based on mild to severe PPHN condition, for example in mild PPHN oxygenation can be given using a nasal cannula, followed by closed monitoring to the response as a consideration whether it is necessary
to increase the oxygen supplementation or not \(^{14,19}\). In this study, it was also known that nine babies received sildenafil without combined with other therapies, with the good outcome. The reason are, it depends on the severity of PPHN itself and as the sample if the baby’s was suffered from mild to moderate PPHN. The severe PPHN mostly tends to give a poor outcome due to the maladaptation and mal-development processes that aggravate the condition, thus condition will needing a therapy including illoprost, surfactants and inotropes \(^{18}\).

Complications during hospitalization suspected can effecting the outcome babies with PPHN. In this study were seen, 13.5% of PPHN infants died followed by sepsis and 21.6% followed by pneumonia. But, even though both of it have a fairly high incidence but there was no association with the outcome. Sepsis and pneumonia can cause high mortality and morbidity in PPHN, but it needs other circumstances conditions such as condition during pregnancy, labor history or perinatal history that could influenced the outcome \(^{12,18}\).

The aim of this study are to become the reference for further research which can be experimental study research to prove the association between of each characteristic, diagnostics and management to the outcome of PPHN.

**Conclusion**

The characteristics of PPHN in dr. Soetomo Hospital consist of, baby boy, with term infant, normal birth weight, perinatal history of asphyxia and MAS, with the complications of pneumonia, maternal history of eclampsia, and delivery procedures by C- section. PPHN could diagnosed with differences in pre- and post-ductal saturation, confirmed by echocardiography followed with the results moderate PPHN. The management of PPHN in dr. Soetomo Hospital was received oxygenation supplementation using mechanical ventilator and \(O_2\) CPAP, with drug combination frequently used sildenafil with inotropic, sildenafil with illoprost and inotropic, and also sildenafil single administration. With the outcome of PPHN were in a cured condition.

**Conflict of Interest :** None declared.

**Acknowledgements :** This work was supported by the staff of the Departement of Pediatrics in Dr. Soetomo General Hospital/Airlangga University, Surabaya.

**Source of Funding:** The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

**Ethical Clearance :** This study was approved by medical researched ethical Health Research Ethics Committee, Dr. Soetomo Hospital Surabaya No. 1845/KEPK/II/2020.

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Factors that affect on the Event of Lung TB in Jati Kudus Health Center

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Abstract

Pulmonary tuberculosis is an infectious disease caused by Mycobacterium tuberculosis, which can attack the lungs and other organs. Pulmonary tuberculosis is still one of the world’s public health problems even though TB control efforts have been implemented in many countries since 1995. The target of the national pulmonary TB prevention program is eliminated in 2035 and Indonesia free from pulmonary TB in 2050. Purpose of Research, this is to find out the factors that influence the incidence of pulmonary tuberculosis in the Jati Health Center Area. This study uses secondary data taken from data from the Jati Health Center and the analysis in this study uses the CHAID Exhaustive method. The results of this study indicate that the number of pulmonary tuberculosis patients with a positive smear suspicion when viewed from the type of sufferers is mostly new cases as many as 216 people. Associated with the type of patient who came for treatment at the Teak Health Center, all patients seeking treatment received a cure category of 99.6% which is a new patient treatment category. So the factors that influence the incidence of pulmonary tuberculosis are the type of patient and treatment results.

Keyword: exhaustive CHAID, tuberculosis, treatment results, type of sufferer

Introduction

Pulmonary tuberculosis is an infectious disease caused by Mycobacterium tuberculosis, which can attack the lungs and other organs. Lung tuberculosis is still one of the world’s public health problems even though TB control efforts have been implemented in many countries since 1995. The national pulmonary TB prevention program targets are eliminated in 2035 and Indonesia free from pulmonary TB in 2050.¹

Data from World Health Organization² states that every year, millions of people in the world have been infected with pulmonary tuberculosis. One of the countries with the most pulmonary TB sufferers in the world is Indonesia. The population reported with the highest cases (43% of the total number of pulmonary tuberculosis cases in Indonesia) is found in the provinces of West Java, Central Java and East Java.³

The notification rate for all cases of pulmonary TB in the population of Central Java Province in 2017 is 132.9 per 100,000 population, this shows that the discovery of pulmonary TB cases in Central Java has increased compared to 2016 which was 118 per 100,000 population.⁴ Data on pulmonary TB cases that obtained from 19 public health centers in the Kudus Regency area in 2014 stated that as many as 495 people were infected with pulmonary tuberculosis.⁵

Method

This study uses secondary data taken from data from the Jati Health Center. The population in this study was all people in the working area of the Jati Health Center who came for treatment at the Puskesmas and conducted sputum checks. While the sample in this study is the entire community in the work area of the Jati Health Center with the incidence of pulmonary tuberculosis.
The analysis in this study used the CHAID Exhaustive method. Exhaustive CHAID is an exploration method for classifying data by building a classification tree that can provide information in the form of independent variables that significantly influence the dependent variable. Exhaustive CHAID has three stages, namely: the merging stage, the splitting stage, and the stopping stage.\(^{(6)}\)

The dependent variable in this study is the incidence of pulmonary tuberculosis. While the independent variables in this study are age, sex, type of patient, and treatment results.

**Result**

**Characteristics of Respondents**

Of the 313 research subjects, their characteristics can be seen in the table below:

<table>
<thead>
<tr>
<th>Number</th>
<th>Karakteristik Data</th>
<th>f</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>72</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>&lt;= 25 Years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 25 Years</td>
<td>241</td>
<td>77.0</td>
</tr>
<tr>
<td>2</td>
<td>Sex</td>
<td>173</td>
<td>55.3</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>140</td>
<td>44.7</td>
</tr>
<tr>
<td>3</td>
<td>Type of Sufferer</td>
<td>299</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td>New sufferer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sufferers relapse</td>
<td>14</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>Treatment results</td>
<td>229</td>
<td>73.2</td>
</tr>
<tr>
<td></td>
<td>Heal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complete Treatment</td>
<td>84</td>
<td>26.8</td>
</tr>
</tbody>
</table>

Independent Variables that Affect the Incidence of Lung TB

**Table 2. Variable Significance Test**

**Model Summary**

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Growing Method</th>
<th>EXHAUSTIVE CHAID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable</td>
<td></td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Independent Variables</td>
<td></td>
<td>Age, Sex, Type of sufferer, Treatment result</td>
</tr>
<tr>
<td>Validation</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Maximum Tree Depth</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Minimum Cases in Parent Node</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Minimum Cases in Child Node</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Results</th>
<th>Independent Variables Included</th>
<th>Treatment result, Type of sufferer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Nodes</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Number of Terminal Nodes</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Depth</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>
The results of the Exhaustive CHAID analysis showed that the independent variables that significantly affected the incidence of pulmonary tuberculosis were the treatment outcome variable and the type of sufferer. While gender and age variables do not affect the incidence of pulmonary TB, so these variables are automatically discarded. The number of nodes formed is 5 (five) pieces, the number of terminal nodes is 3 (three) pieces and the depth of the trees formed there are 2 (two) branches.

Figure 1 shows that from a sample of 313 people studied, the results obtained were 267 patients with positive smear suspected (85.3%) and 46 patients with negative smear suspected (14.7%). The treatment outcome variable is the best predictor variable to explain the incidence of pulmonary tuberculosis, so the variable is used as an insulating variable. The treatment outcome variable is divided into two nodes (nodes 1 and 2), node 1 is the patient whose treatment results is cured. For node 2 is a patient with complete treatment.

At node 1, it is continued by patient type variables which are divided into two nodes (nodes 3 and 4) with a recurrence category (node 3) and a new category (node 4). Whereas on the second node, the partitioning process is stopped because all cases contained in the node have identical values for each predictor variable, so the second node becomes the last node.
The accuracy of the CHAID Exhaustive Method Classification

Table 3. Accuracy of Classification Exhaustive CHAID Methods

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BTA Positive Suspect</td>
<td>BTA Negative suspect</td>
</tr>
<tr>
<td>BTA Positive Suspect</td>
<td>228</td>
<td>39</td>
</tr>
<tr>
<td>BTA Negative Suspect</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>Overall Percentage</td>
<td>73.2%</td>
<td>26.8%</td>
</tr>
</tbody>
</table>

Growing Method: EXHAUSTIVE CHAID
Dependent Variable: Tuberculosis

Table 3 is a classification performance evaluation can be determined by looking at the overall accuracy value generated in the Exhaustive CHAID analysis of 87.2%. The accuracy between observations and predictions for the incidence of pulmonary tuberculosis with a positive smear suspect category was 85.4% with mis-classification errors of 14.6%. While the accuracy between observations and predictions for the incidence of pulmonary tuberculosis with a negative smear suspected category was 97.8% with mis-classification of 2.2%.

Discussion

In this study respondents aged <= 25 years were 72 (23%), while those aged> 25 years were 241 (77%). This shows that in terms of age, respondents in the work area of the Teak Health Center were mostly of productive age. This is consistent with previous WHO reports two-thirds of TB cases occur in the economically productive age group, which is 15 - 59 years. (7)

In this study, respondents who were male were 173 (55.3%), while those who were female were 140 (44.7%). This shows that the average gender of pulmonary TB respondents in the Jati Health Center area is male. Similar to the results of research from Susilayanti et al in BP4 Lubuk Alung, respondents were male as many as 784 (70.8%), while those who were female were 324 (29.2%). (8)

Other studies have shown that men are more susceptible to M. tuberculosis infection. This can be related to greater smoking habits in men, which causes interference with the respiratory system immunity so that it becomes more susceptible to infection. Disturbances in the respiratory tract immunity can be in the form of mucociliary damage due to toxic cigarette smoke and decrease the response to antigens, thereby increasing the susceptibility to pulmonary tuberculosis. (9)

The number of pulmonary TB patients with suspected smear positive when viewed from the type of sufferer mostly in the form of 216 new cases. This is probably due to the number of patients who come to the Puskesmas Jati who have never come for treatment and have never received pulmonary TB treatment. The results of this study are consistent with Karolina’s research at the Kabanjahe Health Center in Karo District that the largest proportion of pulmonary TB sufferers in new cases is 97.5%. (10)

In addition to new cases, the number of pulmonary TB sufferers with suspected smear positive also there were 92.9% recurrence patients. Results This study is in accordance with research Sukmaningtyas, et al (11) that patients who complete treatment with less / more than 6 months (not on time) have a 5% risk of recurrence to
patients who complete treatment exactly 6 months. In a systematic review study, the largest recurrence rate occurred in India, which was 10%, which is the highest among other Countries.\textsuperscript{(12)}

Associated with the type of patient who came for treatment at the Jati Health Center, all patients seeking treatment received a cure category of 99.6% which is a new patient treatment category. The choice of treatment category adjusts to the type of patient and the result of smear examination. Research by Laily et al\textsuperscript{(13)} at the Tuminting Health Center in Manado showed that 194 patients had been treated regularly (99%) and the remaining 2 patients (1%) had irregular treatment. This shows that the awareness of the Tuminting community for treatment and recovery is good. Regularity of treatment is closely related to the results of treatment to be achieved by patients.

**Conclusion**

1. Patients with pulmonary TB who are suspected of having BTA positive who seek treatment at Jati Health Center are mostly of productive age.

2. Patients with pulmonary TB with suspected BTA positive who seek treatment at Jati Health Center are mostly male.

3. Factors that influence the incidence of pulmonary TB in the Jati Health Center area are the type of patient and treatment outcome.

4. In general, the type of pulmonary TB sufferers with the most positive smear suspects found in Jati Health Center are new cases and relapse sufferers.

**Suggestion**

It is necessary to educate the public about the pulmonary TB disease, so that they know the cause of pulmonary TB disease. In addition, the community must also prevent pulmonary tuberculosis by maintaining a healthy environment and promptly taking treatment if there are symptoms of pulmonary TB disease, so it is not transmitted to others.

**Conflict of Interest:** The authors have no conflicts of interest associated with the material presented in this paper.

**Acknowledgements:** On this occasion, the authors would like to thank the Head of Central Java Health Office

**Ethical Clearance:** This research was conducted after obtaining a Certificate of Ethical Worthiness from the Health Research Ethics Commission of the Faculty of Dentistry, Airlangga University Number: number 685/ HRECC. FODM / X / 2019.

**Source of Funding:** Personal Researcher

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Study the Role of pH in Curli Biogenesis Gene Expression in Enterobacter Cloacae Local Isolates

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1Doctor at Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq, 2Doctor at Basic Medical Science Department, Collage of Dentistry, Mustansiriyah University, Baghdad, Iraq

Abstract

Enterobacter cloaeca is the most common pathogenic species of the genus Enterobacter, identified in hospitalized and enfeebled patients as the etiologic agent of many infections and considered as a significant bacterial pathogen in recent years. E. Cloaeca are typical gram negative opportunistic forms of bacteria that cause illness after another infection or injury has influenced the host immune system and are related with nosocomial infections. The infection may be caused by GIT, UTI or cross contamination of the blood. The present study focused on the existence of the main curli biogenesis gene (csgA) and the role of pH as an environmental factor in the gene expression (csgA) for curli biogenesis. In this study, urine samples from 75 patients in Baghdad city clinically diagnosed with urinary tract infection, for gram staining, Api20System and gene expression (csgA) gene in Standardized pH7 and acid pH4 RT-PCR was performed with specific primers. Conventional gram staining techniques, agricultural methods and System API 20E showed good results for E. Cloaeca. For the 75 patients, 10 (13.3%) the findings find the highest gene expression fold values for the (csgA) gene in pH7 while the lowest fold value for (csgA) at acid pH4 (0.1577) thus the modify conditions growth such as pH of bacteria E. Cloaeca triggers gene expression changes for biogenesis process. the findings of rpoB gene expression, which was used as a control gene, indicated that this gene was good as a housekeeping gene.


Introduction

E. Cloaeca is also a common nosocomial pathogen responsible for bacteraemia and lower respiratory, urinary and intra-abdominal infections, as well as endocarditis, septic arthritis, osteomyelitis and skin and soft tissue infections. And endocarditis, septic arthritis, osteomyelitis and diseases of the skin and soft tissue infection. The skin and gastrointestinal tract are the most frequent places where E. Cloaeca can be infected [1]. E. Cloaeca are often isolated from nosocomial infections, including pneumonia, urinary tract and bloodstream infections [2]. Curli is a new class of bacterial surface structures which is expressed in E. Cloaeca, Escherichia coli and Salmonella spp. and is specified by its ability to bind to serum protein fibronectin [3]. Curli Fimbriae is a new class of surface compositions on the surface of E. Cloaeca, E.coli and Salmonella, which are characterized by their ability to bind with protein serum fibronectin [4]. Associated with the adhesion and assembly of bacterial cells on surfaces. As well as being an essential part of the extracellular matrix, that contributes to the formation of the biofilm. The curly cilia is a distinctive element of virulence, because it interacts with a wide range of host proteins, including cell-based intercellular proteins and link-phase proteins, thus helping bacteria

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DOI Number: 10.37506/ijfmt.v15i1.13589
spread to the host. Curly cilia owned toll like receptors, which activates the innate immune system, therefore this considered as a molecular type associated with the pathogen (PAMPs) [5].

**Material and Methods**

**Samples:**

A total of 10 isolates from 75 urinary cultures were collected in this study in patients with UTI admitted to governmental AL-Kathumia pediatric hospitals in Baghdad, these isolates were obtained from April to September 2017. We obtained the sample and stored it. Identification of Bacteria as Enterobacter Traditional biochemical methods (API20 E, Biomerieux, France) were used to conduct cloacae. Bacterial isolates have been maintained for further analysis by inoculation of single pure isolated colony to brain heart infusion agar (BHIA) slant incubated at 37 °C during the night, stored at 4 °C for a few weeks [6,7].

The optimal pH which affected on (csgA) gene expression:

Approximately 0.1 mL of each strain’s nutrient broth culture was applied to 10 mL of BHI broth and incubated at specific pH 4 and 7 with temperature tolerance and 24-hour incubation at 37 °C, Extraction of RNA was done using General RNA Extract-ion Kit (Disbio, China).

Complete RNA extraction with TRIzol:

Complete RNA was collected from all samples using the General RNA Extraction Kit (Disbio, China) using the manufacturer’s pro-protocol. Synthesis of cDNA from mRNA: By Using Wiz ScriptTM RT FDMix Kit, the complete RNA was reversely transcribed to complementary DNA (cDNA). The treatment was conducted in a 20μl reaction volume, as advised by the manufacturer. 20μl was the complete amount of RNA to be reversely transcribed. Thermal cycler steps of cDNA Reverse Transcriptase conditions are shown in Table 1.

<table>
<thead>
<tr>
<th>Table (1): cDNA synthesis program of rpoB and csgA genes for mRNA.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step</strong></td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Real time qRT PCR study the expression of rpoB and csgA genes ∆∆CT

The 2^−DDCt method has been used to compare the transcript levels between various samples [8–10]. The interest gene CT has been normalized to that of the internal control gene. The variance between the rpoB (internal control gene) and the csgA (interest gene) cycle threshold (Ct) values was Analysed by using the qPCR software, QIAGEN Real-time PCR system ((Rotor Gene Q, Germany). The rates of gene expression and the alteration of the fold were quantified by the threshold cycle (Ct) and the ∆∆Ct was used to assess the fold of gene expression.

<table>
<thead>
<tr>
<th>Table (2): Primers used in the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primer sequence</strong></td>
</tr>
<tr>
<td>rpoB F 5’ - GCA ACT TGT TGT CGC GGA TT- 3’</td>
</tr>
<tr>
<td>rpoB R 5’ - TCG ACC GTC GTC GTA AGC T - 3’</td>
</tr>
<tr>
<td>csgA F 5’-ATT GCA ATC GTA GTT TCT GG -3’</td>
</tr>
<tr>
<td>csgA R 5’ATW GAY CTG TCA TCA GAG CCC TGG - 3’</td>
</tr>
</tbody>
</table>
Table (3): Thermal profile of rpoB and csgA gene expression.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95 °C</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95 °C</td>
<td>15 seconds</td>
<td>40</td>
</tr>
<tr>
<td>Anneal</td>
<td>60 °C</td>
<td>60 seconds</td>
<td></td>
</tr>
</tbody>
</table>

Gene expression of csgA in Different environmental factors

Volume equal to 0.1 mL of a nutrient-broth culture overnight of each strain was added to 10 mL of BHI broth, a shaking incubated at different pH. The extraction of RNA complete by RNA extraction kit was carried out in the same steps, then cDNA synthesis for mRNA was completed. RT master Mixed and programs used under standardized conditions.

Results and Discussion

Enterobacter species, especially Enterobacter cloaceae, are important nosocomial pathogens that are responsible for approximately 13.20 percent of UTI Iraqi patients according to previous study [11]. Several surface structures of bacteria, including curli, flagella, pili and exopolysaccharide; Play roles in different aspects of development of biofilms [12]. Biofilms can be a problem in the food industry and in hospital environments Curli allow the adherence of Salmonella enteriditis to Teflon and stainless steel, which can contribute to the creation of biofilms and contamination of surfaces frequently used in the food industry [13], the strain’s ability to form biofilm relied on csgA. These researchers indicated curli were essential during the attachment process in the initial stages of biofilm growth [14]. RpoB rRNA expression has been shown to be strongly dependent on the physiological state of the bacterial cell in bacteria, one of the most widely used housekeeping genes in accordance with gene expression results., The essential principle for the use of housekeeping genes in molecular experiments is that their expression in the cells or tissues under investigation remains constant [15]. Variation of total change in RpoB rRNA expression was analyzed. In various researchers using the 2-Ct value and the 2-Ct ratio, the expression fold for the rpoB gene was 1, 0.933 under variable PH conditions, respectively pH7 and pH 4. Little variability in the gene expression fold makes the gene RpoB a reliable reference gene as shown in (Table 4).

Table (4) Fold expression of rpoB gene for pH factor.

<table>
<thead>
<tr>
<th>factor</th>
<th>Mean ct of rpoB</th>
<th>Factor 2 –Δct</th>
<th>Experimental /control</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control temp.37c°, pH7</td>
<td>30.5</td>
<td>6.5 E.q</td>
<td>6.5Eq/ 6.5 E.q</td>
<td>1</td>
</tr>
<tr>
<td>pH 4</td>
<td>30.6</td>
<td>6.1 Eq</td>
<td>6.1 E.q/6.5 E.q</td>
<td>0.933</td>
</tr>
</tbody>
</table>

RT-qPCR expression for csgA gene
The expression csgA is highly regulated. \textit{E. Cloacae} isolates were efficiency. Biofilm- forming, significantly correlated with the csgA and csgD gene mRNA expression rates. The curli protein fimbriae emerged as tangled fibers and the curli-proficient strain formed mature biofilms \cite{16}. Various environmental factors, including incubation period, incubation temperature and pH-effect on virulence expression \cite{15}. By using Relative quantitative RT- PCR assay, we analyzed the mRNA expression of csgA genes by comparing bacterial growth with different pH factors as a variable factor for each sample, we studied the role of pH on csgA biofilm gene expression. The Ct values of gene amplification were reported from the quantitative RT PCR software. Relative quantification used to measure the gene expression fold change \cite{8}. This depends on the standardization of Ct values determining the rpoB gene, which is the difference between the mean Ct values of each case’s csgA cDNA amplification replica and the rpoB gene.

<table>
<thead>
<tr>
<th>groups</th>
<th>Means of csgA</th>
<th>Means of rpoB</th>
<th>Δ ct</th>
<th>2- Act</th>
<th>Experimental/controls</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>29.28</td>
<td>30.5</td>
<td>-1.22</td>
<td>2.32</td>
<td>2.32 /2.32</td>
<td>1</td>
</tr>
<tr>
<td>pH4</td>
<td>32.05</td>
<td>30.6</td>
<td>1.45</td>
<td>0.366</td>
<td>0.366/ 2.32</td>
<td>0.1577</td>
</tr>
</tbody>
</table>

The results in table (5) showed gene expression folding for the csgA gene by comparing bacterial growth with different pH factors for each sample as variable factor. The gene expression fold in pH4 was in (0.1577) times lower than normal. These findings suggest a substantial decrease in the expression of the csgA gene at pH 4 relative to control (Tm37, pH7). This suggests that acidity is an important factor in bacterial pathogenicity as pH has been identified as a regulator for the expression of virulent genes and is likely to be express curli and Suggest that this virulent factor could play a major role in the pathogenesis and invasiveness of \textit{E. Cloacae} \cite{16}, suggests that curli fimbriae expression in \textit{E. Cloacae} plays an significant role in the development of biofilms Which is the first stage of bacterial pathogenicity.

\textbf{Acknowledgement}: This research was supported by the Department of Genetic Engineering, Institute of Genetic Engineering, and Postgraduate Biotechnology.

\textbf{Ethical Clearance-} Taken from Institute Research Ethics committee.

\textbf{Source of Funding-} Self

\textbf{Conflict of Interest -nil}

\textbf{References}


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Neonatal Death Incidence in Healthcare Facility in Indonesia: Does Antenatal Care Matter?

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Abstract

Despite having carried out maternity care in healthcare facilities, neonatal deaths still occur. A study was conducted to analyze the effect of antenatal care (ANC) on neonatal death incidence in healthcare facilities in Indonesia. The study used the 2017 IDHS data. With stratification and multistage random sampling, 13,104 women aged 15-49 years with live births in the last 5 years, who performed maternity care in the healthcare facility, were sampled. Apart from ANC, other independent variables analyzed were a type of place, age, education, wealth, employment, parity, and complication during pregnancy. The final stage employed a binary logistic regression test. The analysis found that women who made complete ANC visits during pregnancy (≥4 times) were 0.486 times more likely than women who did not complete ANC visits (<4 times) (OR 0.486; 95% CI 0.266-0.887). The results of this analysis inform that carrying out a complete ANC visit is a protective factor for women who perform maternity care in healthcare facilities in Indonesia from neonatal death incidence. Apart from ANC, 2 variables were also found to have a significant effect on neonatal death incidence in healthcare facilities in Indonesia. These two variables are the age group and complications during pregnancy. Meanwhile, education level, wealth status, employment status, and parity proved insignificant. It was concluded that ANC is a determinant of neonatal death incidence in the healthcare facility in Indonesia. Complete ANC visits are a protective factor for women who perform maternity care in healthcare facilities in Indonesia from neonatal death incidence.

Keywords: neonatal death, antenatal care, healthcare, pregnancy care.

Background

Neonatal is the most vulnerable period for a child’s survival. The neonatal period, which is defined by WHO as beginning at birth and ending at the full 28 days of life. Child health services start from the time of pregnancy to childhood, which involves the mother as an inseparable part of the child. The infant mortality rate is an indicator of the quality of child health services in a country. Infant mortality is a deadly incident that occurs in the period from the time the baby is born until the baby is not exactly one year old. In 2016, 2.6 million deaths, or about 46% of all under-five deaths, occurred during the neonatal period. More than 40% of all deaths in children under 5 years of age occurred during the neonatal period1–3.

Indonesia has a high rate of neonatal mortality, the 2017 Indonesia Demographic and Health Survey (IDHS) shows that 15/1000 live births. The figure accounts for almost half of the under-five mortality rate in Indonesia4. WHO reports that there are approximately 7,000 newborn deaths per day with three-quarters of neonatal deaths occurring in the first week, and one-third dying within the first 24 hours of birth5. The current trend is that more than 60 countries will miss the SDG target of
reducing neonatal mortality to at least 12/1000 live births by 2030. About half of them will not meet the target by 2050. Identifying the causes of neonatal mortality is essential for intervening. prevention to decrease infant mortality.

Neonatal mortality can be caused by various factors, including maternal factors, socio-culture, services, and health facilities. The Ethiopian study put the proportion of neonatal deaths in health facilities at 20%. The causes of neonatal mortality included preterm birth 28.58%, birth asphyxia 22.45%, neonatal infection 18.36%, meconium aspiration syndrome 9.18%, respiratory distress syndrome 7.14%, congenital malformations 4.08%.

Previous studies reported that neonatal mortality in health facilities was 13.3% or about 30 deaths per 1000 live births. The causes of death were 60.4% low birth weight (LBW), and 55.8% preterm birth. Similar information was also reported in Ethiopia, which reports the causes of neonatal death are preterm birth, birth asphyxia, and infection. Meanwhile, in Iran, it was reported that the highest causes of neonatal death were preterm birth, LBW, and anomalies.

The causes of neonatal mortality in Southeast Asia, in order from the largest, are premature, asphyxia, congenital abnormalities, and sepsis. In previous studies in Indonesia, several factors were associated with an increased risk of neonatal mortality, namely neonatal complications at birth, lack of maternal knowledge about danger signs for neonates, history of complications during pregnancy, delivery carried out at home. Information on several cases of neonatal death shows several reasons that can be prevented by monitoring during pregnancy by doing ANC. Based on the background description, this study was aimed at analyzing the influence of ANC on neonatal death incidence in a healthcare facility in Indonesia.

Materials and Methods

The study employed secondary data from the 2017 IDHS as analysis material. The 2017 IDHS sample was determined through stratification and multistage random sampling. The analysis unit was women in childbearing aged, 15-49 years old, who had given birth in the last 5 years in a healthcare facility. Several 13,104 women were sampled. The 2017 IDHS has received ethical approval from the National Ethics Committee. All respondent identities have been deleted from the dataset. Respondents have signed and agreed to their involvement in the 2017 IDHS. Utilization of 2017 IDHS data for this research has received permission from ICF through the website: https://dhsprogram.com/data/new-user-registration.cfm.

Neonatal death is death in the neonatal period or the first twenty-eight days of life. ANC was the respondent’s acknowledgment of the amount of ANC utilization during pregnancy. The ANC utilization was divided into 2 criteria, namely <4 and ≥4. Apart from ANC, other independent variables involved in the analysis were the type of place of residence, age group, education level, wealth status, employment status, parity, and complication during pregnancy.

Parity is the number of living children a woman is born with. In this study, parity was divided into two, namely primiparous (<2 children) and multiparous (≥2 children). Complications during pregnancy were the respondent’s acknowledgment of complications experienced during pregnancy until delivery. These problems consist of: prolonged labor, vaginal bleeding, fever, convulsions, baby in the wrong position, swollen limbs, faint, breathlessness, tiredness, and others.

This study conducted 2 stages of analysis. The first stage, performed bivariate with chi-square to analyze the relationship between ANC and other variables involved in the analysis. The second stage, multivariate with binary logistic regression to determine the effect and see the odd ratio of the independent variable to the dependent variable. All stages of analysis employed IBM Statistic SPSS 21.

Findings

Table 1 displays descriptive statistics of neonatal death incidence in a healthcare facility by ANC in Indonesia. It appears that the two categories of ANC are dominated by women who do not experience neonatal death. Based on the type of place of residence, both ANC categories are dominated by women who live in urban areas. Meanwhile, based on the age group, the two ANC categories were dominated by women in the 30-34 age group.
Based on the education level, both ANC categories are dominated by women who have secondary education. Based on wealth status, women who made incomplete ANC visits (<4 times) were dominated by the poorest women. Otherwise, women who have complete ANC visits (≥4 times) are dominated by the richest women. Meanwhile, based on employment status, both ANC categories were dominated by unemployed women. Based on parity, the two ANC categories are dominated by multiparous women. Finally, based on complications during pregnancy, both ANC categories are dominated by women who do not experience a complication during pregnancy.

Table 2 shows the results of the binary logistic regression of neonatal death incidence in a healthcare facility in Indonesia. It appears that women who made complete ANC visits during pregnancy (≥4 times) were 0.486 times more likely than women who did not complete ANC visits (<4 times)(OR 0.486; 95% CI 0.266-0.887). The results of this analysis inform that carrying out a complete ANC visit is a protective factor for women who perform maternity care in healthcare facilities in Indonesia from neonatal death incidence.

Complete ANC visits (≥4 times) according to government recommendations, at least 4 visits during pregnancy, helping pregnant women to monitor and control risks during pregnancy. ANC provides routine monitoring of height and weight gain, identification of maternal or fetal medical problems, counseling on tobacco or substance use, providing psychosocial support, nutritional advice, and early intervention that can reduce adverse pregnancy output, including the occurrence of neonatal death. This finding is in line with previous findings conducted in urban areas in Indonesia.

Apart from ANC, 2 variables were also found to have a significant effect on neonatal death incidence in healthcare facilities in Indonesia. These two variables are the age group and complications during pregnancy. Meanwhile, education level, wealth status, employment status, and parity proved insignificant.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Antenatal Care Visits</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 4 times</td>
<td>≥ 4 times</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Neonatal Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2312</td>
<td>96.3%</td>
</tr>
<tr>
<td>Yes</td>
<td>89</td>
<td>3.7%</td>
</tr>
<tr>
<td>Type of place</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>1384</td>
<td>57.6%</td>
</tr>
<tr>
<td>Rural</td>
<td>1017</td>
<td>42.4%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>64</td>
<td>2.7%</td>
</tr>
<tr>
<td>20-24</td>
<td>371</td>
<td>15.5%</td>
</tr>
<tr>
<td>25-29</td>
<td>652</td>
<td>27.2%</td>
</tr>
<tr>
<td>30-34</td>
<td>683</td>
<td>28.4%</td>
</tr>
<tr>
<td>35-39</td>
<td>439</td>
<td>18.3%</td>
</tr>
<tr>
<td>40-44</td>
<td>168</td>
<td>7.0%</td>
</tr>
<tr>
<td>45-49</td>
<td>24</td>
<td>1.0%</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>25</td>
<td>1.0%</td>
</tr>
<tr>
<td>Primary</td>
<td>502</td>
<td>20.9%</td>
</tr>
<tr>
<td>Secondary</td>
<td>1324</td>
<td>55.1%</td>
</tr>
</tbody>
</table>
By age group, women in the 15-19 age group were 0.210 times more likely than women in <15 age group to experience neonatal death incidence (OR 0.210; 95% CI 0.073-0.608). Women in the 20-24 age group are 0.194 times more likely than women in <15 age group to experience neonatal death incidence in healthcare facilities in Indonesia (OR 0.194; 95% CI 0.069-0.547). Women in the 25-29 age group were 0.222 times more likely than women in the <15 age group to experience neonatal death incidence (OR 0.222; 95% CI 0.076-0.648). Meanwhile, women in the 30-34 age group were 0.268 times more likely than women in <15 age group to experience neonatal death incidence in healthcare facilities in Indonesia (OR 0.268; 95% CI 0.090-0.798). Age group as a determinant of neonatal death incidence was also reported in studies in Tanzania, and Bangladesh19,20.
Continued... Table 2. The result of binary logistic regression of neonatal death incidence in the healthcare facility in Indonesia (n=13,104)

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age: 40-44</strong></td>
<td>0.204</td>
<td>0.369</td>
</tr>
<tr>
<td>Education: No education</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Education: Primary</td>
<td>0.998</td>
<td>1.002</td>
</tr>
<tr>
<td>Education: Secondary</td>
<td>0.896</td>
<td>0.874</td>
</tr>
<tr>
<td>Education: Higher</td>
<td>0.540</td>
<td>0.519</td>
</tr>
<tr>
<td>Wealth: Poorest</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wealth: Poorer</td>
<td>0.073</td>
<td>0.537</td>
</tr>
<tr>
<td>Wealth: Middle</td>
<td>0.229</td>
<td>0.677</td>
</tr>
<tr>
<td>Wealth: Richer</td>
<td>0.750</td>
<td>1.099</td>
</tr>
<tr>
<td>Wealth: Richest</td>
<td>0.682</td>
<td>0.872</td>
</tr>
<tr>
<td>Employment: Unemployed</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Employment: Employed</td>
<td>0.073</td>
<td>1.454</td>
</tr>
<tr>
<td>Parity: Primiparous</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Parity: Multiparous</td>
<td>0.058</td>
<td>1.846</td>
</tr>
<tr>
<td>Complication during pregnancy: No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Complication during pregnancy: Yes</td>
<td>***0.000</td>
<td>2.269</td>
</tr>
</tbody>
</table>

Note: ‘p<0.05; “p<0.01; ““p<0.001.

Based on complications during pregnancy, women who experience complications during pregnancy are 2.269 times more likely than women who do not experience complications during pregnancy to experience neonatal death incidence (OR 2.269; 95% CI 1.500-3.434). This information shows that experiencing complications during pregnancy is a risk factor for neonatal death incidence in healthcare facilities in Indonesia. This finding information reinforces the results of previous studies that found similar results\(^2\text{1-}\text{23}\). It is necessary to strengthen the early identification of obstetric complications and urgent intervention to prevent neonatal mortality\(^2\text{4}\). The results of this analysis again to confirm the importance of quality ANC during pregnancy\(^1\text{7}\).

**Conclusions**

Based on the research results, it could be concluded that ANC is a determinant of neonatal death incidence in healthcare facilities in Indonesia. Apart from ANC, 2 other variables were also proven to be determinant determinants of neonatal death incidence in the healthcare facility, namely age group and complication during pregnancy.

**Acknowledgments:** The author would like to thank the ICF International, who has agreed to allow the 2017 IDHS data to be analyzed in this article.

**Source of Funding:** Self-funding

**Conflict of Interest:** The authors declare no conflict of interest, financial or otherwise.

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Relationship between sexual desire and premenstrual syndrome in young women in Iraq

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Abstract

Sexual desire could be affected by many psychological and physiological aspects. This research aims to find out the relationship between sexual desire and premenstrual syndrome in young female. A Descriptive design cross-sectional study was carried throughout the present study in order to achieve the study objectives. The period of the study is from 20th September 2019 to 28th April 2020, included 200 young married women in the reproductive stage, with ages between (17-31) years. Sexual Desire Inventory 3 (SDI-3) has been used to assess sexual desire after modification to meet the cultural and social considerations. The current study revealed that it explains that the highest percentage of the women subgroup are: women with ages between (21-24) years old (50%), those who the age of their husbands (23-26) years old (46.5%), those who live urban residents (84%), those who they and their husbands work as employee (58.5%), those with barely sufficient monthly income (38.5%), those who are graduated from college or above. The results showed that there is a significant association (p <0.05) between sexual desire of women and each of: backache, dysmenorrhea and mood instability; while there is no significant relationship with each of: (backache, breast tenderness, headache, irritability, sadness, aloneness, bloating, vaginal pain, vaginal dryness). It is concluded that sexual desire may affected by some symptoms of the premenstrual syndrome. Further studies may be needed to confirms these results.

Keywords— premenstrual syndrome, sexual desire, young women

Introduction

Sexual Desire is the operative force in nearly all sexual expression. Desire is closely related to the concept of lust, and at its most powerful it approaches the concept of passion. There are people whose previous experiences may make it difficult to recognize or acknowledge desire (¹). Sexual desire is a conscious longing for sexual activity with the object of desire, resulting in sexual satisfaction. Sexual desire can result in rapid or gradual psychic arousal, the resulting physiological changes (rapid breathing and pulse, lubrication, erection), a need to approach the object of desire, physical contact that brings satisfaction, and the possible release (²). Sexual and reproductive health and well-being are essential if people are to have responsible, safe, and satisfying sexual lives. Sexual health requires a positive approach to human sexuality and an understanding of the complex factors that shape human sexual behavior. These factors affect whether the expression of sexuality leads to sexual health and well-being or to sexual behaviors that put people at risk or make them vulnerable to sexual and reproductive ill-health (³). Reproductive health is a state of complete physical, mental, and social well-being and not merely the absence of disease and infirmity, in all matters relating to the reproductive system and to its

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functions and processes. Reproductive health therefore implies that people are able to have a satisfying and safe sex life and that they have the capability to reproduce and the freedom to decide if, when and how often to do so. The presence or absence of premenstrual syndrome (PMS) complaints have also been used as indirect indicators of hormonal profiles that may affect sexual behavior (4). The current study aimed to investigate the relationship between sexual desire and premenstrual syndrome

Methods

A Descriptive Design Cross-sectional study was carried throughout the present study in order to achieve the study objectives. The period of the study is from 20th September 2019 to 28th April 2020.

A Non-Probability (Purposive Sample) of 200 young married women in the reproductive stage, those who lives in Al-Najaf Al-Ashraf City, Southern of Iraq. The final study instrument consists of three parts:

Part 1: Demographic Data

A demographic data sheet, which consist of (8) items, include: Age of wife, Body mass index, Residential environment, Educational status of wife, Occupational status of wife, Age of husband, Occupational status of husband, Monthly income.

Part 2: Reproductive Health Data

The second part of the questionnaire is consists of (10) items, include: Age at marriage, Marriage duration, Number of children of participants, Status of breastfeeding, Menstrual cycle characteristic, Ovulation, Pregnancies, Mode of delivery, Birth control use, Gynecological History.

Part 3: Sexual Desire Inventory3 (SDI-3).

The researcher has developed Sexual Desire Inventory 3 (SDI-3) version from the original version that was initially suggested by Specter et al. (1996) to meet the cultural and social considerations.

Statistical Analysis

Descriptive statistics presented as mean, standard deviation, frequencies and percentages. Chi-square test was used to compare frequencies. Pearson’s correlation test was used to assess the correlations. Level of significance of ≤ 0.05 was considered as significant difference or correlation.

Results

Table (1) shows statistical distribution of study sample (women) by their socio-demographic data, it explains that the highest percentage of the women subgroup are: women with ages between (21-24) years old (50%), those who the age of their husbands (23-26) years old (46.5%), those who live urban residents (84%), those who they and their husbands work as employee (58.5%), those with barely sufficient monthly income (38.5%), those who are graduated from college or above.

According to table (2) and figure (4.3), The classification of women according to their total scores of sexual desire scale is as follows: (low 7%); (moderate 72%) and (strong 21%).

Table (3) shows relationship between sexual desire of women and their premenstrual symptoms, it shows that there is a significant association (p < 0.05) between sexual desire of women and each of: backache, dysmenorrhea and mood instability; while there is no significant relationship with each of: (backache, breast tenderness, headache, irritability, sadness, aloneness, bloating, vaginal pain, vaginal dryness).
### Table (1) Statistical distribution of study sample (women) by their demographic data

<table>
<thead>
<tr>
<th>Items</th>
<th>Sub-groups</th>
<th>Study group Total = 200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td><strong>Age / Years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-20</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>21-24</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>25-28</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>29-31</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>Husband age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-22</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>23-26</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>27-30</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>31-34</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Residency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><strong>Women Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House wife</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Free Jobs</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Husband Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Worker</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Retired</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Monthly Income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Barely Sufficient</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td><strong>Levels of Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>illiterate</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Read and write</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Institute</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>College and above</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>
Table (2): Classification of women according to their total scores of sexual desire scale

<table>
<thead>
<tr>
<th>No.</th>
<th>Sexual Desire Levels</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Low</td>
<td>14</td>
<td>7.0</td>
</tr>
<tr>
<td>2.</td>
<td>Moderate</td>
<td>144</td>
<td>72.0</td>
</tr>
<tr>
<td>3.</td>
<td>Strong</td>
<td>42</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Table (3): Relationship between sexual desire of women and premenstrual symptoms

<table>
<thead>
<tr>
<th></th>
<th>Chi Square</th>
<th>df</th>
<th>P value Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical Discharge</td>
<td>7.24</td>
<td>2</td>
<td>0.02</td>
</tr>
<tr>
<td>Backache</td>
<td>0.86</td>
<td>2</td>
<td>0.65</td>
</tr>
<tr>
<td>Breast Tenderness</td>
<td>4.9</td>
<td>2</td>
<td>0.086</td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>9.29</td>
<td>2</td>
<td>0.009</td>
</tr>
<tr>
<td>Headache</td>
<td>0.11</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>Weakness</td>
<td>7.17</td>
<td>2</td>
<td>0.02</td>
</tr>
<tr>
<td>Mood Instability</td>
<td>2.54</td>
<td>2</td>
<td>0.28</td>
</tr>
<tr>
<td>Irritability</td>
<td>2.16</td>
<td>2</td>
<td>0.34</td>
</tr>
<tr>
<td>Sadness</td>
<td>1.79</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Aloneness</td>
<td>1.66</td>
<td>2</td>
<td>0.43</td>
</tr>
<tr>
<td>Bloating</td>
<td>0.5</td>
<td>2</td>
<td>0.77</td>
</tr>
<tr>
<td>Vaginal Pain</td>
<td>1.99</td>
<td>2</td>
<td>0.36</td>
</tr>
<tr>
<td>Vaginal Dryness</td>
<td>2.13</td>
<td>2</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Discussion

According to table (1), women with ages between (21-24) years constitute the majority of the sample; with a range between (17-31) years; it means that the present study aims to investigate the young women which represent the typical women at the reproductive age; this age range is usually classified as the (peak reproductive age) according to STRAW +10 (Stages of Reproductive Aging Workshop +10) which is a new staging system for categorizing women reproductive ages (6). The age category of the current study may explain other result that the high percentage of women are graduated from college or above (32.5%), and that the majority of them and their husbands work as employee (58.5%); and they
have somewhat sufficient monthly income (38.5%).

Researchers had experienced difficulties in the measurement of sexual desire; some studies have assessed sexual desire by investigating self-reported behavior such as number of intercourses for a specific time (7). The real attempt to quantitatively measure sexual desire is Kinsey and his colleagues, they described sexual desire as a psychosomatic tension that needs to be released, their measurement relied on the how many times sexual activities with orgasm have been achieved (8). Among the widely used measurement to evaluate female sexual dysfunction, is the Female Sexual Function Index (FSFI), in which six sexual domains related to sexual desire are included (9). Some measurements used one-item Likert scale for assessing sexual desire by asking one cognitive questions (like: have you noticed any changes in sexual desire?), the measure assumed that sexual desire is unidimensional (8). The currently used third version of the Sexual Desire Inventory in this study is designed to assess dyadic sexual desire (i.e. having sexual activity with a partner), so that solitary sexual activity (i.e. masturbation) is excluded; another scale that investigate is dyadic sexual desire only is the Hulbert index of Sexual Desire (HISD) with a score ranges between 0-100 (10).

According to table (2), the results of the present study revealed that (72%) have moderate sexual desire, (21%) have strong sexual desire; this may be due to the age of the studied sample in which the majority of their ages between (21-24) years (table 4.1), this results agrees with those obtained by Zegeye et al. (11) who studied a sample of women with ages between (15-25) years, and found that (43.9%) of them have moderate sexual satisfaction, while (43.6%) had extreme sexual satisfaction.

Table (3) shows that cervical discharge may be associated with moderate to high sexual desire; this result comes in agreement with the previous studies that indicated a significant effect of fatigue and weakness on sexual desire in women during the menopausal transition (16); it is clear that sexual activities require good psychological and physical conditions; so that fatigue, weakness and any body disorders may negatively affect the sexual desire.

**Conclusions**

It is concluded that sexual desire may affected by some symptoms of the premenstrual syndrome. Further studies may be needed to confirms these results.

**Ethical Clearance**: Taken from University of Kufa ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Efficiency of Determination of Elemental Composition of Metals and their Topography in Objects of Biological Origin Using Spectrometers

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Abstract

Bodily injuries, caused by firearms and special non-lethal means, which are provided for law enforcement agencies and special services and they are available to the civilian population as well (pistols for firing ammunition equipped with elastic bullets, stun guns, etc.,) and the consequences of torture using various methods and the traumatic factors that caused by them have to get expert objective assessment. Among other laboratory studies, it is important to determine the characteristics of the composition of chemical elements in objects of biological and non-biological origin by performing X-ray fluorescence spectral analysis using modern spectrometers. The object: to determine the characteristics of the elemental composition of metals and their topography in injuries caused by gunshots and stun gun by conducting X-ray fluorescence spectral analysis using spectrometers “ElvaX CEP-01” and “M4 TORNADO”. Conclusions: the use of spectrometers increases the accuracy and objectivity of expert examinations of injuries caused by firearms and electric shock device as they have a wide range of chemical elements detection in the composition of the products of the shot and the electro tag from sodium to uranium. Using X-ray fluorescence spectral analysis, it is possible not only to detect metals in the layers of soot on injuries, but also to conduct a targeted “microscopic” study of their topography for partial group identification of firearms and the installation of electrode metal, which acted as a contact body conductor. X-ray fluorescence spectral analysis is a non-destructive research method.

Key words: gunshot wound, electric trauma, X-ray fluorescence spectral analysis.

Introduction

Terrorism, local wars and internal conflicts, in which law enforcement officers act as one of the parties, are becoming more and more serious problems for all countries of the world. 4, 11, 16 This leads to a wide range of damages caused by the action of weapons on the standard equipment of certain law enforcement agencies, including an action of so-called “non-lethal weapons”, such as means of shock and trauma, equipped with elastic bullets, electric shock devices and more. Situations of excessive use of force and torture, in which firearms and electrical devices can act as a traumatic factor have a special significance in these conditions. 2, 15 Therefore, due to consistently significant number of injuries caused by firearms, special non-lethal means, which are provided for law enforcement agencies and special services and they are used in torture and they are available for locals, it is important to make assessment of the damage of objects of biological origin objectively with the use of highly efficient laboratory equipment, in particular, modern spectrometers.

Many countries around the world use highly sensitive spectrometers based on the use of physicochemical methods of analysis to determine the factors that accompany a shot from a firearm or the consequences of an electric shock device: atomic absorption
spectrophotometry (AAS), mass spectrometry with bound plasma (ICP-MS), atomic emission spectrometry (AES) or neutron activation analysis (NAA). In many cases, the instruments are equipped with scanning electron microscopy in combination with X-ray fluorescence microanalysis (SEM-EDX).

As practice shows, among many factors that characterize the features of gunshot wounds, detection of metals from bullets, shell casings, barrels of firearms formed during firing, and components of powder charge combustion, which are defined as “factors accompanying the shot” or “shot products” are provided sufficiently complete information.3, 12, 14, 17

It is essential to use the methods of elemental analysis and evaluation of the electrotag, which can act as an additional method of research that confirms the mechanism of damage, and even to some extent it indicates the electrode material that caused the damage.7, 8, 13, 18

Detection of chemical elements of metals in the composition of the shot products, electric tags and their identification is performed in the forensic laboratory by a set of methods and techniques known since the twentieth century. These are X-ray fluorescence analysis 4, 10, spark mass spectrometry, atomic absorption analysis, neutron activation analysis, flame emission photometry, emission spectrographic method, infrared spectrometry, etc.1, 4, 5, 19, 21, 22. The methods are highly sensitive and can detect almost the full range of chemical elements. One of their significant disadvantages is the preparation of the sample, which is accompanied by the inevitable destruction and loss of the object of study.

It should be noted that X-ray spectral analysis is a fairly effective and non-destructive method of studying metals from the fragments of explosive devices, bullets, shell casings, barrels of firearms in the composition of the products of the shot and electric tags.9 Its essence is the X-ray irradiation of the object of study and the detection of the spectral composition of secondary radiation, which reflects the quantitative and qualitative indicators of the chemical elements of the object that is under a study.

However, conducting X-ray fluorescence spectral analysis using modern spectrometers is insufficient way to get information about the qualitative and quantitative composition of metal elements and their topography in objects of biological origin, formed as a result of gunshots and electric shock devices.

The object of this paper: to determine the characteristics of the elemental composition of metals and their topography in injuries caused by gunshots and electric shock devices by conducting X-ray fluorescence spectral analysis using spectrometers “ElvaX CEP-01” and “M4 TORNADO”.

**Materials and Methods**

The archival material of the Department of Forensic Medicine of the Kyiv City Clinical Bureau of Forensic Medical Examination was used in the work number 15 “Expert Conclusions” on bodily injuries from shots fired from short-barreled weapons (pistols) and 12 “Expert Conclusions” regarding electric shock devices in cases of domestic and industrial electric injuries with a known contact electrode.

The material of the study in cases of gunshot wounds were 25 pieces of skin and 5 fragments of skull bones removed from the areas of gunshot wounds in people who have died being shot with 9 mm bullets from pistols. The distance of the shot ranged from 30-50 cm.

The study of chemical elements, formed in objects of biological origin due to the gunshot wounds, was performed by X-ray fluorescence spectral elemental analysis on a spectrometer “M4 TORNADO” company Bruker (Germany) using a package of standard analytical techniques.

Pieces of skin and fragments of skull bones with perforated fractures, which were removed from the areas of gunshot wounds in people who being killed by 9mm bullets from pistols, were placed in the working chamber of the spectrometer, where a vacuum pump created a pressure of 20 mbar. Using autofocus, the samples were translated into the focal plane. In the video images of skin flaps and fragments of skull bones with wounds, the scan plane was set, and the horizontal scan lines consisted of 600 points. As a result, the spectra of P, S, K, Ca, Fe, Cu, Zn and Pb. were obtained from the scanning plane of the wound area. Subsequently, the detected elements were mapped in the areas of wounds and perforated
fractures on fragments of skull bones. Photo illustrations were made using a personal computer with a Pentium-4 processor, followed by production of prints.

The materials of the study in cases of electric shock devices were 12 flaps of skin with electrogamps, selected from fatal cases of electric shock devices with known contact electrodes through which the transmission of electrical energy to the human body starts to occur. All 12 cases were selected, among others, taking into account the “material of the contact electrodes”, due to which there was an electric shock (a typical metal wire with a predominance of copper in the chemical composition). The study of chemical elements, formed in objects of biological origin due to damage by electric shock device, was performed by X-ray fluorescence spectral elemental analysis on a spectrometer “ElvaX CEP-01” (country of manufacture is Ukraine). Before the study, flaps of skin with electrogamps were exposed to low temperature in the freezer (-20°C) in order to preserve. After thawing at room temperature and calibration of the spectrometer, skin flaps were placed in the working chamber of the spectrometer. As a result, the spectra of Fe, Cu, and Ni were obtained from the damage sites. The analysis of the obtained data was performed using the integrated RStudio system. Analysis of variance was used to determine differences in the concentration of elements. The boundaries of the differences were determined using the Tukey’s criterion. The distribution of residues was checked for normality using the Shapiro-Wilk test. The standard methods of variation statistics were used in the work.

Results and Discussion

In the study of skin flaps and fragments of skull bones removed from the areas of gunshot wounds in people who were killed by shots fired from 9 mm pistols at close range, the obtained maps of the distribution of elements showed an increased content of iron, copper, zinc and lead, which topographically in maximum concentrations were located at the edges of the wound of the skin (Fig. 1) and the entrance of the skull (Fig. 2) and around them in the form of irregularly shaped belt.

Fig. 1. Maps of distribution of iron (Fe), copper (Cu), zinc (Zn) and lead (Pb) on the edges of the wound on a piece of skin in the area of a gunshot wound due to a 9 mm bullet.
Fig. 2. Distribution maps of iron (Fe), copper (Cu), zinc (Zn) and lead (Pb) along the edges of the wound on a fragment of the skull bone in the area of the perforated fracture after a shot with a 9 mm bullet.

Subsequently, the detected elements in the wound area were mapped on skin flaps, for this purpose, a scan area was set on the object under study. On the received maps of elements distribution, the increased content of iron which in the maximum concentrations is located on edges of elements of damage on skin flaps was noted.

Thus, when applying X-ray fluorescence spectral analysis on the surface of the scan area on skin flaps in the areas of the entrance gunshot wound and on fragments of skull bones in the areas of perforated fracture was revealed elevated levels of iron, copper, zinc and lead, which were unevenly distributed at maximum concentrations on edges of wounds and perforated fractures in the form of irregularly shaped belt.

Detection and targeted “microscopic” study of the topography of chemical elements of iron (Fe), copper (Cu), zinc (Zn) and lead (Pb) in the layers of soot at the edges of the entrance gunshot wounds and in areas of perforated skull fractures caused by shots of 9 mm bullets, allowed not only to detect metals on injuries, but also to conduct a partial group identification of firearms, therefore, identify the weapon itself.

Among the twelve flaps of skin examined in the case of electric trauma, in six cases the electrotags had the appearance of an area of exfoliated dry epidermis of gray color. In two more cases, similar lesions with a slightly greenish tinge were identified. The crater-like form of damage was observed in three cases, and in one observation the damage had the appearance
of a superficial elongated abrasion. In the study of the elemental composition of metals on flaps of leather with electrodes caused by electric current through electrodes with a predominance of copper in their composition, using a spectrometer “ElvaX CEP-01”, the following distribution of trace elements in the area of damage in percentage (Table 1).

**Table 1. Distribution of the elemental composition of metals on 12 pieces of leather with electrical tags (%)**

<table>
<thead>
<tr>
<th>Skin flaps</th>
<th>Metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron (Fe)</td>
</tr>
<tr>
<td>№1</td>
<td>5.558</td>
</tr>
<tr>
<td>№2</td>
<td>3.883</td>
</tr>
<tr>
<td>№3</td>
<td>7.513</td>
</tr>
<tr>
<td>№4</td>
<td>5.418</td>
</tr>
<tr>
<td>№5</td>
<td>4.458</td>
</tr>
<tr>
<td>№6</td>
<td>5.998</td>
</tr>
<tr>
<td>№7</td>
<td>5.913</td>
</tr>
<tr>
<td>№8</td>
<td>5.009</td>
</tr>
<tr>
<td>№9</td>
<td>6.885</td>
</tr>
<tr>
<td>№10</td>
<td>5.040</td>
</tr>
<tr>
<td>№11</td>
<td>5.813</td>
</tr>
<tr>
<td>№12</td>
<td>5.875</td>
</tr>
</tbody>
</table>

Distribution of the elemental composition of metals on 12 pieces of leather with electrical tags (%).

After statistical processing of the obtained data, a normal distribution of values with p.value 0.5417 was established, with a significant predominance in the elemental metal composition of the areas of electrical labels of copper content, the average value of which was 85.72417% with a standard measurement uncertainty of 0.474046.

Our own experience has shown that X-ray fluorescence spectrometers “M4 TORNADO” by Bruker (Germany) and “ElvaX CEP-01” (Ukraine) are successfully used recently in Ukraine. They are used to determine the qualitative and quantitative composition of metal elements and their topography in objects of biological origin, formed as a result of shots from firearms and the action of electric shock devices. This opened new opportunities for laboratory diagnosis of gunshot wounds, weapons and ammunition, as well as damages investigations caused by electric shock devices. The results obtained by us make one deepen and supplement the previously known scientific and practical research. 3, 9, 12, 14, 17
When using X-ray fluorescence spectral analysis in cases of electric shock device applying, opens up opportunities not only to confirm the effect of electric shocker on the body, but also determine the conformity of the metal composition in the area of damage to the metal composition of the electrode, which may be important in establishing circumstances and conditions of injury. Thus, in our study, the constancy and conformity of the metal elemental composition in the areas of electric shock damage to the contact electrode metal was confirmed.\textsuperscript{6, 20}

**Conclusions**

The use of spectrometers increases the accuracy and objectivity of expert examinations of injuries caused by firearms and electric shock devices, as they have a wide range of detection of chemical elements in the composition of the products of the shot and the electric tags from sodium to uranium. Using X-ray fluorescence spectral analysis, it is possible not only to detect metals in the layers of soot on injuries, but also to conduct a targeted “microscopic” study of their topography for partial group identification of firearms and the installation of metal electrode, which acted as a body conductor. X-ray fluorescence spectral analysis is a non-destructive research method, as it does not involve the preparation and study of a control sample.

**Ethical Clearance:** Ethical clearance was obtained from the ‘Ethics Committee’ of the Institution prior to the start of the study.

**Source of Funding:** Self

**Conflict of Interest:** No

**References**


Association of Pure Tone Audiometry with DPOAE in Students after Shooting Practice at East Java State Police Academy, Mojokerto, Indonesia

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Abstract

Objective: Analyzing the association between pure tone audiometry and distortion of otoacoustic emission (DPOAE) in students after shooting practice.

Methods: This study was conducted at the East Java State Police Academy, Mojokerto, Indonesia, from August 2018 to January 2019. Participants were examined for pure tone audiometry and DPOAE two weeks after shooting training. Participants previously had undergone shooting training for 5 months, with schedule of 4 times per week. The statistical test used an independent t test or Mann Whitney test with \( p < 0.05 \).

Results: All participants were men, with an average pure tone audiometry value of 27.50 ± 10.43 dB. The results of pure tone audiometry examination found that most participants had normal hearing (58.00%). This finding was inversely proportional to the results of DPOAE examination, in which most participants had “Refer” (78.00%). The mean pure tone audiometry value on participants with DPOAE “Refer” was 20.36 ± 3.47 dB and DPOAE “Pass” was 29.51 ± 10.87 dB (\( p = 0.008 \)). Most participants had “Refer” DPOAE with normal pure tone audiometry as much as 36.00%, followed by participants with “Refer” DPOAE but mild hearing loss of pure tone audiometry as much as 24.00% (\( p = 0.002 \)).

Conclusion: There is a significant association between pure tone audiometry with DPOAE. Many participants have normal audiometry but “Refer” DPOAE, and followed by participants experiencing mild hearing loss with “Refer” DPOAE.

Keywords: acoustic trauma, pure tone audiometry, DPOAE, police

Introduction

Patients with acoustic trauma often have abnormal distortion product otoacoustic emission (DPOAE) with normal audiometric examination (1). A routine pure tone audiometry examination for shooting athletes cannot predict the risk of hearing loss. Patients with average audiometry have experienced hearing loss. This often causes the athletes to have hearing loss and late treatment(2).

Acoustic trauma is a clinical condition of persistent or permanent hearing loss after exposure to impulses or loud sound waves (3, 4). Acoustic trauma causes organic ear damage due to very large sound energy. Cochlea injury occurs due to excessive physical stimulation in the form of very large vibrations that damage hair cells. In repetitive exposure, damage does not only occur due to physical processes, but also chemical processes in the form of metabolic stimuli that excessively stimulate hair cells that results cells dysfunction which leads to a temporary hearing loss. Damage to hair cells can also result in permanent hearing loss (5).
Hearing loss caused by acoustic trauma often results in permanent disability in army and police personnel \(^{(4)}\). Exposure to firearms eruptions, both large and small caliber, can cause acoustic trauma. Noise from gunfire eruptions, including pure impulsive noise with light gun eruption, has intensity ranging from 150 to 190 decibels (dB). Exposure to high-intensity sound can cause excessive vibration in tympanic membrane that leads to a risk of tympanic membrane perforation. The vibration is then continued to the middle ear through hearing bone that results in a risk of dislocation and damage to hearing bone. The vibration is then delivered to the perilymph and endolymph to vibrate the basilar membrane. This will cause stimulation of hair cells on tectorial membrane excessively that results in reversible hair cell damage. Repeated acoustic trauma can cause hair cells to atrophy and permanent damage \(^{(6)}\).

Based on the description above, this study aimed to determine the association of pure tone audiometry examination with DPOAE in students after shooting practice at the East Java State Police Academy.

**Methods**

Participants of this study were students of the East Java State Police Academy of 2017/2018. Participants should meet the inclusion and exclusion criteria of the study. Inclusion criteria included active participants in shooting training for 5 months and had intact tympanic membrane. Exclusion criteria included patients experiencing ear infections (otitis media), patients experiencing flu and tympanic membrane perforation. Participants have received an explanation regarding the objectives, benefits, rights, and obligations of the participant during the research process. Participants who were willing to take part in the research first filled in informed consent.

This research was conducted at the East Java State Police Academy, Mojokerto, Indonesia, from August 2018 to January 2019. This study employed a cross-sectional design with a total sampling method. The number of participants in this study were 50 participants. The researchers conducted an ethics test at the ethics committee Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, prior to the study. Participants were examined for participant characteristics, pure tone audiometry, and DPOAE. The examination was carried out after the participants had joined shooting exercises for at least 5 months, and the exercises were carried out 4 times a week. Pure tone audiometry and DPOAE examinations were performed two weeks after shooting practice.

Pure tone audiometry is a subjective hearing examination to determine the degree of hearing loss with an audiometer (Interacoustics AD226 Clinical Audiometer; Assens, Denmark). The examination was carried out in a soundproof room at the East Java State Police Academy, Mojokerto, Indonesia. The room’s noise level was 40 to 45 dB. Pure tone audiometry check started at 1kHz 40 dB. If the participant did not respond, the frequency was raised by 5 dB until the hearing threshold was obtained, and the participant responds was reduced by 10 dB. The valued frequency of air conduction was at frequencies of 250Hz, 500Hz, 1kHz, 2kHz, 4kHz, and 8kHz. The evaluated bone conduction frequencies were 500Hz, 1kHz, 2kHz, and 4kHz. The results were grouped according to severity based on the International Standard Organization (ISO) and the American Standard Association (ASA), with the following categories: normal (<25 dB), mild degree (26-40 dB), moderate degree (41-60 dB), severe degree (61-80 dB), and profound (>81 dB).

DPOAE examination is a hearing examination that can detect damage to outer cochlear hair cells using the AuDX-I Bio-logic tool (Natus Medical Inc; CA, USA). DPOAE occurs when two pure tone stimuli with different frequencies are given simultaneously (f1 and f2). Previously set DPOAE filtering parameters include SPL 65/55 dB stimulus intensity levels for lower f1 frequencies (L1) and higher f2 frequencies (L2). The overall passing criteria are DPOAE for the difference in noise level of 6 dB for three of the four f2 test frequencies (f2 of 5, 4, 3, and 2 kHz) \(^{(7)}\). DPOAE is normal if “Pass”, and declared abnormal if “Refer”. Participants were asked to sit in a comfortable chair, and the probe was placed in the participant’s ear canal. Participants were encouraged to be calm and not move their heads during DPOAE recording \(^{(8)}\). This test took around 1 - 5 minutes in ideal conditions, with optimal test techniques \(^{(9)}\).

This research had been declared feasible to be carried out because it passed ethical approval from The Ethics Committee Dr. Soetomo General Academic
The results of the study were presented in the form of mean ± standard deviation (SD) or median (Quartile 1 - 3) and percentage (%). Statistical analysis used an independent t test or the Mann Whitney test (95% CI). Static analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). Statistical test results were significant if \( p < 0.05 \).

**Results**

**Characteristics of Participants**

The participants age ranged from 18 years to 21 years, and most students aged 20 years (42.00%). All students were male. There were participants who experienced a momentary hearing loss (10%). Most participants experienced momentary tinnitus as much as 96.00%. Most participants experienced acoustic trauma as much as 56.00% (Table 1).

**Audiogram Results**

The average audiogram examination results for each frequency were as follows: 250Hz at 26.60 ± 10.47 dB, 500Hz at 26.50 ± 8.64 dB, 1kHz at 25.00 ± 8.33 dB, 2kHz at 28.50 ± 11.44 dB, 4kHz at 31.10 ± 14.15 dB, 6kHz at 26.40 ± 14.88 dB, and 8kHz at 25.70 ± 14.92 dB. The average audiogram results were 27.50 ± 10.43 dB. Audiometry examination results showed that some participants experienced mild to moderate hearing problems. Most participants had an audiometric value within the normal limit (58.00%; Table 1).

**DPOAE Results**

The results of DPOAE examination were as follows: Pass and Refer, where most participant had Refer result (78.00%), while 22% of participants were in “Pass” category (Table 1).

**Association of Pure Tone Audiometry with DPOAE**

This study found a significant difference between the results of pure tone audiometry examination with DPOAE, with a mean participant value with DPOAE “Pass” and “Refer” at 20.36 ± 3.47 dB and 29.51 ± 10.87 dB, respectively (\( p = 0.008 \)). The results of pure tone audiometry results were higher in the “Refer” DPOAE group compared to “Pass” group (\( p < 0.05 \)). The examination results of each frequency could be seen in Table 2.

The pure tone audiometry examination results were categorized into three. Most participants had a normal result (58.00%). Most DPOAE examination results belonged to “Refer” category as much as 78.00%. Most students had “Refer” DPOAE but with normal pure tone audiometry (36.00%), and followed by participants with “Refer” DPOAE but with mild pure tone audiometry in pure tone audiometry results (24.00%; \( p = 0.002 \); Table 3).

**Table 1. Characteristics of participants**

<table>
<thead>
<tr>
<th>Variables</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>4 (8.00)</td>
</tr>
<tr>
<td>19</td>
<td>18 (36.00)</td>
</tr>
<tr>
<td>20</td>
<td>21 (42.00)</td>
</tr>
<tr>
<td>21</td>
<td>7 (14.00)</td>
</tr>
<tr>
<td>Momentary hearing loss</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (10.00)</td>
</tr>
<tr>
<td>No</td>
<td>45 (90.00)</td>
</tr>
</tbody>
</table>
Continuation from Table 1. Characteristics of participants

<table>
<thead>
<tr>
<th>Momentary tinnitus</th>
<th>Yes</th>
<th>48 (96.00)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>2 (4.00)</td>
</tr>
<tr>
<td>Hearing disorder</td>
<td>Normal</td>
<td>29 (58.00)</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>12 (24.00)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>9 (18.00)</td>
</tr>
<tr>
<td>DPOAE</td>
<td>Pass</td>
<td>11 (22.00)</td>
</tr>
<tr>
<td></td>
<td>Refer</td>
<td>39 (78.00)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of pure tone audiometry frequency distribution with DPOAE

<table>
<thead>
<tr>
<th>Audiogram</th>
<th>DPOAE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pass (n=11)</td>
<td>Refer (n=39)</td>
</tr>
<tr>
<td>250Hz</td>
<td>19.55±8.50</td>
<td>28.59±10.19</td>
</tr>
<tr>
<td>500Hz</td>
<td>21.36±7.10</td>
<td>27.95±8.56</td>
</tr>
<tr>
<td>1kHz</td>
<td>18.64±5.04</td>
<td>26.79±8.23</td>
</tr>
<tr>
<td>2kHz</td>
<td>21.36±5.45</td>
<td>30.51±11.96</td>
</tr>
<tr>
<td>4kHz</td>
<td>23.18±8.14</td>
<td>33.33±14.75</td>
</tr>
<tr>
<td>6kHz</td>
<td>17.27±4.10</td>
<td>28.97±15.82</td>
</tr>
<tr>
<td>8kHz</td>
<td>16.36±3.93</td>
<td>28.33±15.82</td>
</tr>
<tr>
<td>Peru tone average</td>
<td>20.36±3.47</td>
<td>29.51±10.87</td>
</tr>
</tbody>
</table>

DPOAE = distortion product otoacoustic emission; *significant 0.05

Table 3. Correlation between DPOAE and pure tone audiometry

<table>
<thead>
<tr>
<th>DPOAE</th>
<th>Pure Tone Audiometry</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL Normal</td>
<td>HL Ringan</td>
</tr>
<tr>
<td>Pass</td>
<td>11 (22.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Refer</td>
<td>18 (36.00)</td>
<td>12 (24.00)</td>
</tr>
</tbody>
</table>

HL=hearing loss; *significant <0.05
Discussion

This study found a significant association between pure tone audiometry and DPOAE in students of East Java State Police Academy, Mojokerto, Indonesia. Police is a job with a risk of being exposed to excessive noise especially during shooting practice (10). At the moment, weapons shooting training is capable of producing noise around 132-165 dB with a frequency spectrum between 150 to 2500 Hz. Noise exposure can cause acoustic trauma. If the condition lasts for a long time, it can cause police to become a group that is at risk of hearing loss (11). Acoustic trauma will trigger damage to hair cells in cochlear organ. Hair cells are circular structures that will vibrate by acoustic signals of sound. Such mechanical vibrations are converted into electrical waves in the auditory nerve (1).

Acoustic trauma causes damage to basal hair cells due to high intensity noise exposure at high frequencies, whereas apex damage is caused by high intensity noise at low frequency. Differences in damage to basal and apex hair cells can be caused by tonotopic differences in the basal area to the cochlea apex, such as the condition of outer hair cell viability, vascularization, intrinsic susceptibility of basal hair cells to free radicals that make basal receptors more sensitive to damage. Antioxidant levels in basal are lower than in the apex, so that damage due to high frequencies is more easily occur in basal part (12).

Damage to outer hair cells makes DPOAE not appear even if given acoustic stimulation. Some cochlea can produce sounds spontaneously as internal sounds are processed and then amplified. Recording sound in cochlea requires a combination of earphones and microphones that can deliver vibrations of sound that enter cochlea and are returned to tympanic membrane that functions as a stethoscope. The installed probe must be impermeable so as to prevent outside sounds enter the ear (13). Damage to cochlear hairs was identified by the DPOAE examination, in which most of participant had “Refer”. This condition was supported by results of pure tone audiometry, showing a higher DPOAE “Refer” compared to DPOAE “Pass” at each frequency. This condition is in accordance with the existing theory, in which acoustic trauma is difficult to be identified only by using audiometry but also by using DPOAE as confirmation. A short-term examination audiometry examination cannot optimally describe the condition of participant’s hearing condition with acoustic trauma disorders. However, pure tone audiometry can give a picture of decreased hearing threshold after acoustic trauma. Thus, both methods have a significant association in examining patients with acoustic trauma.

Conclusions

This study found a significant association between pure tone audiometry and DPOAE. Most participants have normal audiometry but DPOAE “Pass”, and followed by mild hearing loss and DPOAE “Refer”.

Funding: None
Conflict of Interest: The authors declare that they have no conflict of interest.

References


Pharmaceutical Mini-Tablets Overview

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Abstract

Oral dosage forms are considered as the most preferred dosage forms to various age groups of patients. However, conventional solid dosage forms (tablets and capsules) have been associated with some issues; such fluctuation in the plasma drug concentration and swallowing difficulty for some patients. This advocates the need for the continuous development and improvement of tablets and capsule to enhance therapeutic efficacy and increase patient acceptance. Mini tablets technology has been developed to minimize these problems. It aims to facilitate oral administration with minimal swallowing difficulty (especially in pediatrics and geriatrics) and deliver a therapeutic agent, selectively and effectively, to be targeted in a certain position in the body. The most benefit of this technique is the ease of production with facilitated control of stability problems. The size and shape uniformity, with smooth surface area, make minitablets attractive to be incorporated with different medications for the efficient treatment of chronic diseases. This overview outlines properties, production requirements, formulation options and evaluation methods of mini tablets.

Keywords: Mini Tablets, multiple unit dosage forms, compressed mini tablets.

Introduction

Oral solid dosage forms have several advantages compared to other dosage forms. This includes; high patient contentment to administer the dosage, ease of transport, as well as the pattern of drug release, which can be comfortably managed and facilely controlling of stability. Using conventional solid dosage forms, for example: capsules or tablets can cause fluctuation in the plasma drug concentration; thus reducing the therapeutic efficacy. Increasing the dosing frequency decreases patient’s compliance and may end up with an adverse or toxic effect. In order to decrease fluctuation in the concentration, single unit or multi-unit dosage forms with varied release profiles are improved to obtain the desired pharmacological effect. In single unit systems, the controlled drug release is achieved utilizing membrane or matrix systems. In multi-unit systems (mini tablets), drug dose is divided into small subunits. Problems of conventional tablets may be solved using mini-tablets system. Mini tablet technology aims to deliver a therapeutic agent effectively with minimal swallowing difficulty (especially in pediatrics and geriatrics) and reduced dosing frequency; and therefore the maximum therapeutic effect will be obtained as well as a minimum of possible adverse effect. Additionally, a controlled drug delivery system can be developed using mini-tablets systems for the selective and effective treatment of chronic diseases (Figure 1).
Drug delivery systems aim to deliver a therapeutic agent, effectively and selectively, to a specific site in the body; thus minimizing dosing frequency, improving treatment efficiency and reducing potential toxicity. Conventional dosage forms have failed to achieve this and they have been associated with inconsistent plasma drug levels, which may be ineffective or toxic levels[1]. Multi-unit dosage forms have shown the ability to conquer this obstacle, especially when the ingredients represent synergistic or additive impact, and reduce the therapeutic doses into a single unit dosage form. This advocates the advantage of preparing mini tablets dosage units to enhance the therapeutic efficacy and reduce potential side effects. Multi-unit dosage forms have greater bioavailability compared to single units due to the higher credible dissolution profile[2]. Figure 2 shows a comparison among the characteristics of multi- and single- unit dosage forms[3].
**The definition, characteristics, and processing apparatus of mini-tablets**

Mini tablet is an expression used to describe a tablet with small diameter, 3 mm or less (Figure 3). Mini tablets are described for many pharmaceutical applications, such as oral, controlled and targeted drug delivery systems. They can be loaded in capsules, sachets or pressed in large tablets\[1, 4\]. Different dosages and sizes of Mini tablets are generally manufactured easily utilizing rotary tablet press machines with various punches\[1, 2\].

![Figure 3: Comparison of the diameters of conventional tablets and mini tablets](image1)

The usage of multiple punches is advantageous as it shorten the filling time and thus reducing the possibility of powder segregation. The benefits of using multiple punches are presented in Figure 4\[4, 5\].

![Figure 4: Advantages of multiple punches usage in mini tablets production.](image2)

Multiple punches are available as multi-part associated or as monoblocks (Figure 5). Multi piece assemblies exhibit low risk of contamination and they are classified into two types; the first one is the internal cap fixing (Figure 5-1), which is immobilized into the punch body. The second type is the external cap fixing (Figure 5-2). The internal fixing contains fewer pins as well as provides comfortable disassembly and mounting compared to the external one. Monoblock punches (Figure 5-3) are simpler to clean and require less installation time.
Additionally, they are stronger and more resistant against breakage and abrasion compared to multiple punches. The eroded edges can be easily renewed in multiple punches without any requirement to renew the punches. The improper installation is the main cause of eroding and punches damaging. The elevated ratio of length/diameter of the punches makes them nondurable to non-axial stresses. The device speed and length/diameter ratios should be adjusted carefully to minimize this risk. Mini tablets require fewer pressures compared to normal tablets. The process should begin with minimum pressure rates due to the durable diameter of a single punch, which is approximately 2-3 mm and may elevate to 2-3 kN axial force\cite{4}. Figure 6 presents the main advantages of mini tablets\cite{3, 6}.

**Figure 5: Types of staples used in mini tablet production**

Formulation and production requirements

Mini tablets have different parameters compared to conventional tablets. These parameters include; cylindrical hole length, die diameter, size distribution, particle size, length-width ratio as well as bulk and tapped densities. The most important characteristics of mini tablets are the cylindrical hole length and die diameter. There is a proportional relationship between diameter size and bulk flow rate, if diameter is large (4 mm) therefore the bulk flow rate increases, and vice versa. The cause of the flow rate differences is the elevated negative pressure gradient in punch. The environmental factors, such as temperature and humidity, must be taken into account during processing\cite{7}. It is very important to achieve good and reproducible followability, in order to ensure uniformity of content and die filling.

**Figure 6: Advantages of mini-tablets**

During tablet manufacturing, powders should be provided with mechanical resistance to facilitate the process of coating and capsule filling; this resistance can be obtained via good selection of formulation components such as binders and lubricants. Additionally, particle size plays an important role in mechanical resistance. A study carried out in 1998 by Lennartz and Mielck to improve the compact ability of paracetamol powder...
mixtures, they discussed the role of tablet content, size and pressure on capping tendency and tensile strength. It was found that particle size reduction has increased the mechanical resistance and decreased capping tendency, which can be explained by the elevated ratio of surface area/volume in mini tablets compared to that in conventional tablets. Therefore, increasing amount of mixture will increase the friction in punch and die wall; thus leading to obtain a homogeneous distribution of densities[8, 9].

**Formulation types of mini-tablets**

Mini tablets can be formulated into compressed mini tablets, encapsulated mini tablets and biphasic drug delivery systems[1]. Mini tablets can be pressed into tablets or loaded within capsules (Figures 7-1 & 2).

**Compressed mini tablets**

Mini tablets can be easily proceeded into tablets; this will save the high cost of hard gelatin capsules. There are several characteristics of mini tablets, which make them more attractive than pellets and granules such as low porosity, smooth shapes, good mechanical resistance, uniform particle size and smooth surfaces. Release profile of mini tablet can be comfortably modified according to the external phase characteristics, which provide filling of the cavity. Biphasic drug delivery systems are improved via employing several release properties. The first phase in this system works for rapid action and immediate release, whereas the second phase sustains the drug release, which maintains continuous action and decreases dose frequency[1, 10, and 11].

**Encapsulated mini tablets**

Encapsulated coated mini-tablets (Figure 7-2) are developed due to their great ability to improve therapeutic efficacy, patient compliance and dosage regimen. Multifunctional systems, composed of gelatinized hard capsule of minitablets, can be developed such as Rapid-release Mini-Tablets (RMTs), Sustained-release Mini-Tablets (SMTs), Pulsatile Mini-Tablets (PMTs), and Delayed-onset Sustained-release Mini-Tablets (DSMTs) [12, 13].
Tablet coating

Coating is the final step of tablet manufacturing and it has a role in cost elevation (Figure 8). The advantages of coating tablets are illustrated in Figure 9. Tablet coating includes four operations; coating via sugar, film coating, coating with pressure and enteric coating.

Many factors determine the importance to carry out the coating step, such as tablet core strength, the cost of coating material and the desired therapeutic effect[1]. It is complicated to manage the release pattern within the matrix systems in mini tablets because of the high surface area to volume ratio. A study was performed to examine the film-coated matrix mini-tablets for the extended release of a water-soluble drug. It is discussed the role of theophylline-containing mini matrix tablets and non-matrix tablets using different concentrations of ethyl cellulose, and they concluded that it is able to extend the release of water-soluble drugs from mini matrix tablets employing a convenient quantity of film coating[14].

Compressed mini tablets as biphasic drug delivery systems
The production of mini tablets as compressed tablets can reduce the production cost significantly compared to filling them within capsules. It is possible to merge among immediate and controlled release in the biphasic drug delivery systems, the immediate release provides rapid action at the beginning, whereas the controlled release ensures the continuous action in constant rate for certain period of time. Biphasic systems can be modified to be fast - slow or slow –fast\[15, 16\]. The correlation between weight of the mini tablets and amount of powder that will surround them is very important to be considered, this ratio is recognized to be at least 3/1. Filling the gap among mini tablets requires high amount of powder, if small amounts are used, this will end up with fracture after compressing\[2\].

**Mini tablets and modified drug delivery**

To modify the release of active ingredient from dosage form, several techniques can be used, for example: delayed release, targeted drug release, prolonged release as well as pulsatile and bimodal release\[4\].

**Extended release mini tablets**

In this system, the active substance is gradually released for a long time. This can be achieved via extending transition time over the gastrointestinal tract or by modifying the drug diffusion from dosage form. The slow release in extended release tablets can be obtained via changing the dissolution and diffusion of the drug over barrier coating, or matrix system\[17\].

Using lipid excipients is important to achieve extended release in tablets, hydrophobic substances follow diffusion principle (Fickian release) and hydrophilic substances follow diffusion and erosion principles.

(Non-Fickian release). The release of extended-release mini tablets will be slow, increasing system hydrophobicity will slow down the drug release, hydroxypropyl methyl cellulose (HPMC) is a hydrophilic polymer, which performs an important role in forming a resistant or less permeable hydrogel layer\[18,19\].

Drug solubility plays a critical role in the release profile. Weakly acidic and basic drugs exhibit pH-dependent solubility. This type of solubility tends to alter the ionic or non-ionic drug ratios according to pH in the release medium or the gastrointestinal fluid. In pharmaceutical production of extended release dosage forms, it is good to achieve solubility which is pH independent\[20\]. In order to obtain pH independent solubility from pH dependent solubility, microenvironments should be created using a pH modifying substance. Immediate and extended release profiles can be easily controlled by pH modifiers.

If salt ingredients are introduced in the formulation of an immediate release dosage forms, it will decrease the dissolution of less soluble compounds. To achieve a pH-independent release for the extended release of weak acidic or basic drugs, pH modifiers may be introduced, which can be combined with a basic drug for example to enhance the solubility significantly at high pH values\[21\].

**Pulsatile and bimodal release**

Fluctuations in drug concentration can occur because of the physiological factors (heart rate, blood pressure, hormone, enzymes and plasma proteins) and circadian rhythms in pathological situations. To solve this problem, several drug delivery systems are produced\[22\]. To obtain a Chrono therapeutic effect, pulsatile drug release is used to delay release in a programmed manner. This system is known as a time controlled system\[23\]. Pulsatile drug delivery is considered as a perfect choice for the treatment of diseases, which require a chronotherapy principle (such as bronchial asthma and angina pectoris). Pulsatile release is obtained using a controlled releasing polymer for coating tablets, which acts as a protective layer. Depending on the drug physicochemical characteristics, the release happens at certain times\[24, 25\]. Pulsatile release coatings may be rupturable, erodible, permeable and semipermeable film coating (Figure 10).
In case of multiple release profiles, pulsatile release systems should be utilized, as in elevated metabolism with first pass effect and pharmacologically tolerated drugs. The advantages of drug administration in divided doses are reduction of bacterial resistance as well as enhancement of biological tolerance\cite{26}.

Pulsatile release prevents the possible interaction between the dosage forms and the gastrointestinal tract\cite{27}. Coating a drug core with functional polymers results in multiple releases from pulsatile system (Figure 11).

**Figure 10: Performance of oral coated drug delivery systems with pulsatile release**

*Figure 10: Performance of oral coated drug delivery systems with pulsatile release*

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Pulsatile release prevents the possible interaction between the dosage forms and the gastrointestinal tract\cite{27}. Coating a drug core with functional polymers results in multiple releases from pulsatile system (Figure 11).

*Figure 11: Multilayer coated pellets*
Bimodal drug delivery systems exhibit several release properties within a single unit to enhance therapeutic efficacy and patient tolerance. It is possible to have multi delivery systems such as rapid, prolonged, extended and delayed release systems. The drug release rate in zero order kinetic system is independent of blood concentration; this system is recognized as a perfect condition to maintain the desired amount of drug in the blood. The absorption rate varies with different digestive parts; it is usually slow in the stomach, too slow in the distal part of the gut and high in the proximal part of the gut. This means that drug release rate should be changeable according to site of action to obtain a constant drug plasma concentration. Bimodal systems provide such a volatile release\[28, 29\].

**Floating mini tablets targeted to the gastrointestinal system**

Drug absorption can be increased using floating systems in the stomach, which extend the drug’s residence time. This system is beneficial for drugs, which have poor solubility and/or stability problem at intestinal pH. It is also beneficial for drugs act locally in stomach and it can decrease the possible local irritation that may occur as an adverse effect for certain drugs\[30, 31\]. Medicines that have narrow absorption windows, low solubility at intestinal pH and high absorption rate in the stomach as well as the ones act locally in the stomach are considered as good candidates to be prepared as floating drug delivery systems. The density of this system is lower than that of the aqueous medium of the gastrointestinal tract (usually less than 1 g / ml) and therefore capable to float at the surface of the stomach fluid. Floating systems are classified into two subtypes: effervescent and non-effervescent systems and they can be single- or multi-units\[31,32\]. Matrix-forming polymers are employed in non-effervescent systems such as polysaccharides and hydrocolloids. The result of adding these polymers is that the system swells once subjected to stomach fluid, whereas protecting the integrity shape. The drug release is controlled via the air, which introduces the swollen polymer and permits floating to occur. The external fluid introduces the dosage via swelling the system and permits drug dissolution. After that, the dissolved drug diffuses through the hydrated gel layer\[33\].

**Mucoadhesive mini tablets**

Mucoadhesive systems are advantageous to achieve local and systemic effect. This system permits prolonged drug existence at the active site; thus increasing the local effect and enhancing the therapeutic efficacy\[34, 35\]. Bioavailability can be increased by introducing mucoadhesive polymers, which adhere to the gastric mucosa surface; thus increasing drug residence in this site for long time\[36\]. Highest mucoadhesive power had been shown with using thiolate polymers; they raised bioavailability more than penetration enhancers\[37, 38\].

**Evaluation of mini tablets properties**

Evaluation of powder mixture is carried out using three measurements; bulk density, tapped density and powder compressibility (Carr’s index and Hausner ratio).

**Bulk density**

It is the ratio of powder mass to the bulk volume of untapped powder; it involves the inter-particulate void volume. Many factors influence the bulk density, such as the powder density and the arrangement of the interstices among the particles in the powder bed. Measurement of bulk density is very sensitive, fine shaking of dust mass can alter density. The bulk density of a certain weight is discussed largely in the American Pharmacopoeia, using different methods (graded cylinder, volumetric method and a container measurement). The bulk density is usually represented by g/ml or g/cm\(^3\). In case of powder weight expressed by M and the initial powder volume is expressed by V\(_0\), bulk density is represented as: M/ V\(_0\). Calculating the average of at least three separated measurements is usually performed to have a representative correct value.

**Tapped density**

It is the ratio of powder mass to the volume occupied by the powder after it had been tapped for a certain time. After determining the initial powder volume, compression of powder is taking place. In case of powder weight expressed by M and the compressed final powder volume is expressed by V\(_F\), Tapped density is represented as: M/ V\(_F\). Calculating the average of at least three separated measurements is usually performed to have a representative correct value.
Measurement of the compressibility of powders

Powder flow and batch characteristics are affected by possible particles interaction. Information about powder flow and particle interactions can be obtained from the bulk and tapped densities. This comparison is performed using the Compressibility Index (Carr’s Index) and Hausner Ratio (Table 1). The Compressibility Index and Hausner’s Ratio can be calculated using the following formulas\[^{[39]}\]:

- Compressibility index: \(100\left(\frac{V_0 - V_F}{V_0}\right)\)
- Hausner’s Ratio: \(\frac{V_0}{V_F}\)

### Table 1: Flow properties and Compressibility Evaluation of Powders

<table>
<thead>
<tr>
<th>Compressibility Index (%)</th>
<th>Flow Properties</th>
<th>Hausner Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\leq 10)</td>
<td>Excellent</td>
<td>1.00-1.11</td>
</tr>
<tr>
<td>11-15</td>
<td>Good</td>
<td>1.12-1.18</td>
</tr>
<tr>
<td>16-20</td>
<td>Available</td>
<td>1.19-1.25</td>
</tr>
<tr>
<td>21-25</td>
<td>Acceptable</td>
<td>1.26-1.34</td>
</tr>
<tr>
<td>26-31</td>
<td>Poor</td>
<td>1.35-1.45</td>
</tr>
<tr>
<td>32-37</td>
<td>Very poor</td>
<td>1.46-1.59</td>
</tr>
<tr>
<td>&gt; 38</td>
<td>Very very poor</td>
<td>&gt; 1.60</td>
</tr>
</tbody>
</table>

Mini Tablets control

Weight Variation

Depending on European Pharmacopoeia, 20 randomly samples are selected and weighed, then the weight average is calculated, percentage variation can be observed in up to two of these weights but it must not vary by more than twice that percentage (Table 2).

### Table 2: Weight Variance Evaluation

<table>
<thead>
<tr>
<th>Pharmaceutical Form</th>
<th>Average Weight</th>
<th>Percent Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>80 mg or less</td>
<td>10</td>
</tr>
<tr>
<td>(Uncoated and Film Coated)</td>
<td>80-250 mg</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>More than 250 mg</td>
<td>5</td>
</tr>
<tr>
<td>Capsules, Granules</td>
<td>Less than 300 mg</td>
<td>10</td>
</tr>
<tr>
<td>(coated and uncoated)</td>
<td>More than 300 mg</td>
<td>7.5</td>
</tr>
<tr>
<td>and Powders</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Uniformity of tablets

To ensure uniformity of content (uniformity of active pharmaceutical ingredients (API) in each tablet); thus obtaining the desired therapeutic effect without loss of efficacy or any possible toxic effect. There are two methods to recognize the uniformity of tablets: weight variation or content uniformity. The drug’s contents are examined to define if the single contents are in limits; Content Uniformity (CU) and weight variability (MV) tests of various dosage forms are illustrated in Table 3.
Table 3: Content Uniformity and Weight Variability Tests for Dosage Forms

<table>
<thead>
<tr>
<th>Dosage Form</th>
<th>Type</th>
<th>Subtype</th>
<th>Dosage and proportion of active substance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥ 25 mg and ≥ %25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤ 25 mg and ≤ %25</td>
</tr>
<tr>
<td>Tablets</td>
<td>Uncoated</td>
<td>-</td>
<td>MV</td>
</tr>
<tr>
<td></td>
<td>Coated</td>
<td>film coated</td>
<td>MV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CU</td>
</tr>
<tr>
<td>Capsules</td>
<td>Hard</td>
<td>-</td>
<td>MV</td>
</tr>
<tr>
<td></td>
<td>Soft</td>
<td>Suspension, Emulsion, Gel</td>
<td>CU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution</td>
<td>MV</td>
</tr>
<tr>
<td>Solids in single dose container</td>
<td>One-component</td>
<td>-</td>
<td>MV</td>
</tr>
<tr>
<td></td>
<td>Multi-components</td>
<td>freeze-dried solution</td>
<td>MV</td>
</tr>
<tr>
<td>Liquid in single dose container</td>
<td>-</td>
<td>-</td>
<td>MV</td>
</tr>
</tbody>
</table>

**Friability**

This experiment is carried out under specific situations to confirm the existence of lamination or fractures in uncoated tablet or any surface damage. It is performed once and if there is any fracture or crack in a sample, this sample is considered to fail the friability test\(^{[39]}\).

**Dissolution test**

It is performed to define the dissolution rate of the active pharmaceutical ingredients (API) in solid dosage forms (capsules or tablets). Many factors should be recognized to prepare a sample for this test:

The utilized device, rotating speed, sampling method, analysis method and amount of (API) required to be dissolved\(^{[39]}\).

**Disintegration test**

It is utilized to confirm if the solid dosage form (tablet or capsule) are dispersed in a specific time, when they are subjected to a liquid medium, under the test situations demonstrated in Figure 12\(^{[40]}\).

![Figure 12: the conditions of disintegration occurrence](image-url)
Mini-Tablet Drug Delivery System for Pediatric Dosage Form (PDF)

Pediatric have a problem of swallowing difficulty, and therefore the most prescribed dosage form for them is the liquid dosage form which provides ease of administration. Because of the lack of stability of the liquid drugs and inaccurate dosing intake; mini-tablet technique provides the facility of delivering drugs for pediatrics in an accurate dose as well as in high stability in a comparison with the bulk dosage form[41].

Few drugs commercially available as minitablets, for examples: capsules of Propafenone HCl as antiarrhythmic, Sachet of Terbinafine HCl as Antifungal, capsules and sachet of Sodium Valproate for epilepsy, capsules of Fenofibric acid for cholesterol and Stick Pack of Ivacaftor for Cystic Fibrosis (CF)[41].

Conclusion

Pharmaceutical mini-tablets provide several benefits compared to conventional tablets, which make them as excellent alternatives for granules and pellets. They have low porosity and high mechanical strength. They can be formulated into tablets or filled in capsules. Mini-tablets can be used to deliver incompatible drugs for the effective treatment of different chronic disorders. It enhances the therapeutic efficacy and patient compliance. As discussed in this review article, mini tablets technology represents a promising field, which requires high interest from pharmaceutical researchers because of the wide therapeutic applications. As such, mini-tablets seem best implemented for small volume, high value products, particularly for pediatric patient populations that would benefit by this unique dosage form.

Acknowledgements: The authors are grateful to the Philadelphia University, Amman, Jordan for the financial support granted to cover the publication fee of this paper.

Conflict of Interest: The authors declare they have no conflicts of interest to disclose.

Authors’ contributions

MB organized the project and the article writing. HS is a student under MB supervision who prepared the manuscript. HS and MS contributed to the writing style and proof reading. All authors have read and approved the manuscript.

Ethical Clearance: Not applicable.

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Relationship Between High Sensitivity TNF-α WITH clinical outcome During Admission In Acute Ischemic Stroke

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Abstract

Background: Tumor necrosis factor alpha (TNF-α), together with other pro-inflammatory cytokines such as Interleukin-1b (IL-1b), IL-6 and IL-8, play a role in the ischemic injury of the central nervous system. TNF-α has adverse effects on ischemic brain tissue. The mechanism generated is a multicomplex process. In other studies, an increase in TNF-α can actually have neuroprotective effects. This study aims to determine the relation between the levels of the High Sensitivity TNF-α with clinical outcome in acute ischemic stroke.

Method: Thirty patients with acute ischemic stroke patients who admitted in Dr. Soetomo hospital Surabaya during the period December 2011 to February 2012. 2 cc of vein blood for examination High sensitivity TNF-α was drawn during admission from acute ischemic stroke. Clinical outcome in acute stroke was measured by NIHSS score.

Results: From 30 research samples, the mean age of patients was 59.53 ± 11.51 years. The mean High Sensitivity TNF-α level in the study subjects was 2 ± 0.99 pg / ml. NIHSS examination showed the median value of the NIHSS was 5 with a range of 2-19. There is a correlation but not statistically significant with the strength is very weak between High Sensitivity TNF-α with the NIHSS in patients with acute thrombotic stroke (r = 0.100 and p = 0.600).

Conclusion: There is relationship between serum levels of High sensitivity TNF-α with clinical outcome in acute ischemic stroke patients.

Keywords: TNF-α, HS TNF-α, clinical outcome, acute ischemic stroke.

Introduction

At present, stroke is ranked as the second leading cause of death worldwide. In the first year after a stroke, about 20% of patients die.¹ The number of ischemic stroke sufferers is greater than hemorrhagic stroke. In western countries, the number of ischemic stroke sufferers is around 80-85% of all stroke sufferers. Ischemic stroke is caused by a decrease in cerebral blood flow resulting in death and brain cell dysfunction.² Other problems are associated with the risk of infection in stroke patients.³

Tumor necrosis factor alpha (TNF-α), together with other pro-inflammatory cytokines such as Interleukin-1b (IL-1b), IL-6 and IL-8, play a role in the ischemic injury of the central nervous system. Some experimental studies have shown that TNF-α has adverse effects on ischemic brain tissue. The mechanism generated is a multicomplex process.⁴ In other studies, an increase in TNF-α can actually have neuroprotective effects.⁵

The problem that arises is whether there is a correlation between serum TNF levels and neurological deficits that occur in patients with acute thrombotic stroke. NIHSS is a score frequently used in the evaluation of stroke clinical outcome.⁶

Method

This research is an analytic study with cross sectional design. Inclusion criteria include: Patients with acute thrombotic strokes, having suffered a thrombotic stroke 4 hours until the 7th day and are willing to sign informed consent. Exclusion criteria include: Having an infection before an attack (eg, injury, heat), having acute
ischemic heart disease, having a history of malignancy, metabolic disorders (electrolytes, liver function and kidney function), using immunosuppressive drugs, NSAIDs. Based on the formula for calculating the correlation sample, the minimum sample size is 16.97 rounded up to 30 people.

All study subjects underwent the same clinical and laboratory examinations. High Sensitivity TNF-α serum examination was carried out in the Prodia Surabaya laboratory which was carried out by the ELISA-Sandwich method. The normality of data distribution was examined by the Saphiro Wilk test. Spearman’s statistical test was used to determine the correlation between the two variables that were not normally distributed.

**Results**

This study involved a total sample of 30 acute ischemic stroke patients who came to the Emergency room Dr. Soetomo hospital who met the inclusion criteria and did not meet the exclusion criteria. General characteristics and laboratory tests are shown in Table 1.

### Table 1 General Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (66,7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10 (33,3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>59.53 ± 11.51</td>
<td>35 – 80</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td>162.67 ± 25.18</td>
<td>110 – 210</td>
</tr>
<tr>
<td>Dyastolic blood pressure (mmHg)</td>
<td></td>
<td>96.33 ± 14.96</td>
<td>60 – 140</td>
</tr>
<tr>
<td>Onset of attack (days)</td>
<td></td>
<td>3.4 ± 1.57</td>
<td>1 – 7</td>
</tr>
</tbody>
</table>

**Serum TNF-α levels in research subjects**

The mean High Sensitivity TNF-α level in the study subjects was 2 ± 0.99 pg / ml. The mean High Sensitivity TNF-α levels can be seen in table 2.

### Table 2 Mean High Sensitivity TNF-α levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Sensitivity TNF-α levels (pg/ml)</td>
<td>2.00 ± 0.99</td>
<td>0.55 – 4.79</td>
</tr>
</tbody>
</table>

**NIHSS results in research subjects**

The results of the NIHSS examination showed the median value of the NIHSS was 5 with a range of 2 - 19, this data can be seen in table 3.

### Table 3 Average NIHSS values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS</td>
<td>5</td>
<td>2 – 19</td>
</tr>
</tbody>
</table>
Correlation between High Sensitivity TNF-α levels with NIHSS value

A positive correlation was obtained with the strength of a very weak correlation between High Sensitivity TNF-α levels and NIHSS values and not statistically significant with \( p = 0.600 \) and the correlation coefficient of 0.100. This can be seen in table 3.

### Table 3 Correlation between High Sensitivity TNF-α levels and NIHSS values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Sensitivity TNF-α levels vs NIHSS values</td>
<td>0.100</td>
<td>0.600</td>
</tr>
</tbody>
</table>

**Discussion**

The design of this study was cross sectional. Selection of consecutive sampling as a method of selecting research subjects because this method is the best non-probability sampling and easy to do.\(^7\)

During approximately 4 months of research, 30 subjects were found to meet the research criteria. Of these 30 subjects, 20 (66.7\%) of them were male and 10 (33.3\%) were female. The ratio between male and female subjects is 2:1 (table 1). This research shows that men experience more acute thrombotic strokes than women. In accordance with the epidemiological data of stroke, men suffer more strokes than women.\(^8\,9\) Other literature studies state that the percentage of thrombotic strokes in women in India and Southeast Asia is 33\% to 36\%.\(^10\)

From 30 research samples, the mean age of patients was 59.53 ± 11.51 years (table 1). In accordance with the epidemiological data of stroke that many stroke suffered by patients aged over 45 years.\(^10\,11\) Baseline data that has been collected is then tested for normality first with the Kolmogorov-Smirnov test (KS test). This test aims to determine the distribution of normal or abnormal data.\(^12\)

Analysis using the KS test found that the distribution of the study data was normal. Therefore an analysis to determine the correlation between High Sensitivity TNF-α and NIHSS levels in acute strokes was performed using Pearson correlation analysis.

A positive correlation was seen with a very weak correlation strength of 0.100 between High Sensitivity TNF-α and NIHSS levels in patients with acute stroke, which was not statistically significant at \( p = 0.600 \). This means that the higher the High Sensitivity TNF-α level, the greater the NIHSS value, but not statistically significant.

The results of this study are supported by other studies\(^13\) which state that the increase in TNF-α in liquor fluids and serum of patients in acute stroke has a correlation with the severity of stroke as measured by the SSS (Scandinavian Stroke Scale) and BI (Barthel Index). In addition there are other studies that show a TNF-α correlation with stroke outcomes using mRS (modified Rankin Scale) and BI (Barthel Index) in the first week of stroke.\(^14\)

**Conclusions and Recommendations**

There is a positive correlation with the correlation coefficient \( r = 0.100 \) between High Sensitivity TNF-α and functional expenditure as measured by NIHSS in acute ischemic strokes even though it is not statistically significant \( p = 0.600 \). Further research is needed to measure High Sensitivity TNF-α levels with NIHSS 1, 7 and 30th day of stroke sufferers. So that it can be known the actual neurological outcome level, as well as with a larger sample size so that it can provide more representative results.

**Conflict of Interest:** There is no conflict of interest among the authors.

**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee.

### References

3. Handayani IA et al. The Impact of High Cortisol Level in Increasing Incidence of Infection Cases


Study the effect of purified alginate from *Pseudomonas aeruginosa* on Hep G2 and A549 cell lines.

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**Abstract**

Alginate was extracted from highest alginate producer of *P. aeruginosa* 29 isolate, it was determined by biochemical method (Carbazole-borate assay) and molecular method (PCR), where the amplified *algD* gene samples showed that clear bands at one level for all the produced isolates and molecular weight were 313 on the leader scale. Partially purified of alginate using gel filtration chromatography (Sephadex G-200) showed the present of single peak and initial alginate fraction at 17 to last fraction 28 of alginate concentration, where the highest alginate concentration was at fraction 23. The cytotoxic effect of different concentrations of purified alginate on cancer cells (HePG2, A549) and normal cells WRL68 was tested. The result of half-maximal inhibition concentration (IC50) values of purified alginate treated WRL68 and HepG2 cells after 24, 48, 72 hours of incubation at 37ºC were (187.8, 62.0, 83.07) μg/ml, (102.3, 42.5, 61.03) μg/ml as respectively. While, the IC50 of purified alginate treated WRL68 and A549 cells after 24, 48, 72 hours of incubation at 37ºC were (126.2, 63.57, 90.31) μg/ml, (117.8, 57.06, 65.7) μg/ml as respectively.

**Key words:** *P. aeruginosa*, Alginate, cytotoxicity

**Introduction**

Alginate is one of the few polymers produced by some prokaryotic and eukaryotic organisms. The marine algae is mainly the eukaryotic source of alginate. Moreover, amongst the prokaryotes, two bacterial genera *Azotobacter* and *Pseudomonas* are recognized to including some species ability to produce alginates (1). Bacteria produce a wide range of extracellular polysaccharides to adapt to and thrive in diverse niches of the environment. Many of these polymers have attracted great attention due to their implication in biofilm formation, capsule formation, virulence, or for their potential medical. One important exopolysaccharide, alginate, is produced by various *Pseudomonas spp.* and *Azotobacter vinelandii*. Alginate is of particular interest to cystic fibrosis patients because of its role in the pathogenesis of *Pseudomonas aeruginosa* lung infection (2). These polysaccharides have a chemical structure composed of subunits of (1–4)-β-d-mannuronic acid (M) and its C-5 epimer α-l-guluronic acid (G) (3). *Pseudomonas aeruginosa* is responsible for many nosocomial infections as a common opportunistic pathogen. It converts to a mucoid phenotype after infection of the lungs of patients with cystic fibrosis (CF), characterized by overproduction of the exopolysaccharide alginate. Alginate not just protects the pathogen against antibiotics and host immune defenses, but can also block the lungs of the patient. Such infections are extremely difficult to remove and always contribute to the patient’s death (4, 5). Extensive applications are outcome of alginate rheological properties, as well as from biocompatibility, biodegradability and toxicity absence. Their pharmacological and biological properties, such as antioxidant activity, antimicrobial ability, anticancer and immune stimulatory properties and strong free radical scavenging, have led to the advancement of alginate research’s in the biomedical field (6, 7).

**Material and Methods**

**Collection of samples.**
In this study, 180 samples were collected from clinical cases including both males and females with different ages, from Al-anbar teaching Hospitals and general Al-amiriyah hospital. Bacterial identification was achieved by gram stain, Cultural characteristics, biochemical tests and vitek2 technique (8).

**Alginate extraction** was done according to the method mentioned by Pedersen et al., (9) The precipitated alginate washed twice with isopropanol and re-suspension with normal saline(0.9%). the alginate concentration of this suspension was determined by the Carbazole-borate assay (10).

**Molecular determination of algD gene.**

The primer forward sequence (5’to3’) algD-F was CGTCTGCGCGAGATCGGGGT and the primer reversed sequence (5’to3’) algD-R was GACCTGACGGTGCTCGGA, it was designed according to (11), the algD gene was determined by polymerase chain reaction (PCR) through Master Mix, Primer and the reaction program showed in following table :

**Table (1 ): PCR program for alg D gene amplification**

<table>
<thead>
<tr>
<th>Steps</th>
<th>(°C)</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95</td>
<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>45 sec</td>
<td>35</td>
</tr>
<tr>
<td>Annealing</td>
<td>60</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>60 sec</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>7</td>
<td>7 min</td>
<td>3</td>
</tr>
</tbody>
</table>

**Purification of alginate;** Sephadex G-200 was prepared as recommended by Pharmacia Fine Chemicals Company. A quantity of Sephadex G-200 was suspended in 0.01 M Tris-HCl buffer, pH 8, degassed, and packed in a glass column (1.5x20 cm), then equilibrated with the same buffer. Five ml of extracted alginate solution obtained from the extraction step was applied on to the column. Elution was achieved at a flow rate of 0.5 mL/min and the same buffer was used for equilibration. Eluted fractions (4ml) were collected and assayed for uronic acid content by carbazole-borate assay of alginate fraction by measuring the absorbency at (525 nm).

**Cytotoxicity assay:** The assay of MTT was used to determine the cytotoxic effect of purified alginate concentrations on cellular lines, Hepatoblastoma-derived cell line (HepG2) , human cell lung cancer (A549), with normal cell line (WRL68) , where purified alginate concentrations were 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml at 37 °C during 24, 48, 72 hours(12,13).

**Results and Discussion**

**Isolation and identification:** In this study, 180 samples were collected from clinical cases including both males and females with different ages, where, collected from different sources; wounds, burns, abscess, cystic fibrosis, ear infection, vaginal infections and urinary tract infections. The isolation results showed that the number of samples showing positive bacterial growth on the used media was 125 samples (69.45%), while 55 samples (30.55%) of total samples did not demonstrate bacterial growth. Alginate production; All 25 bacterial isolates obtained from different sources were have investigated ability it’s to produce alginate. The results of the study showed 13 (52%) ps. aeruginosa isolates were producer of alginate ,while ps. aeruginosa isolates were 12 ( 48%) not producer. In order to select the efficient isolate in the production of alginates, the
capability of these thirteen isolates were examined in the alginate production medium by determining the concentration of crude alginate in the culture medium following the isopropanol precipitation. The results indicated local isolate of *Ps. aeruginosa* 29 (ps.a29) yielded higher alginate production (123.6 µg/ml). From these findings it was determined that *Ps. aeruginosa* 29 was the most active in alginate production because it was isolated from the cystic fibrosis tissue and alginites are required in this tissue to invade the tissue. Because of the importance of alginate in the formation of biofilms and also in resistance of antibiotics and phagocytosis (14). This results agreed with Al-Janabi, (15) that also reported different concentrations of produced alginate from *ps. aeruginosa* isolates and detect higher alginate production from cystic fibrosis isolate. Detection of algD by PCR; The results of the electrophoresis of the amplified gene algD samples showed that clear bands at one level for all the produced isolates and molecular weight were 313 on the leader scale. While the isolated samples were not produced, the algD gene bands did not appear at any level except for one isolate that showed one algD gene band as silent gene not expressed of phenotype, it shown in figure(1). The reason may be to suppression of same algD gene or the others genes responsible for alginate formation; like, alg44 gene is role important of alginate biosynthesis, Alg44 has a cytoplasmic PilZ domain that binds to the c-di-GMP cofactor and is needed to activate the Alg44 function and to alginate production. Point mutations in the PilZ domain lead to the loss of c-di-GMP binding which results in the loss of alginate production (16).

**Figure(1): Agarose gel electrophoresis for 60 min (2 % agarose, 75 V/cm2) of amplified algD gene (313bp)**

Purification of alginate; Extracted alginate of highest alginate production by local isolates of *P. aeruginosa* (Pa29) was partially purified using gel filtration technique. For this purpose 5ml of extracted alginate solution was added to sephadex G-200 column and eluted by Tris-HCl buffer Eluted sample were collected as fractions 4 ml with flow rate 0.5 ml/min and assayed for uronic acid by carbazol borate assay to detect alginate positive fraction, the results in figure(2) showed the percent of single peak and initial alginate fraction at 17 to last fraction 28 of alginate concentration, where the highest alginate concentration was at fraction 23 and used in the cytotoxicity study.
Cytotoxic effect of purified alginate on Hep G2 and A549 cell lines: Various concentrations of purified alginate determined its toxic effect on cellular lines Hepatoblastoma-derived cell line (HepG2), human cell lung cancer (A549), with normal cell line (WRL68). The assay of MTT was used to determine the cytotoxic effect of purified alginate on these cancer cell lines. Where those concentrations were 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml at 37°C during 24, 48, 72 hours. In graph pad prism, data analyzes conducted in µg/ml and log values of µg/ml were plotted using log (Inhibitor) versus normalized response Curve. For the most significant values of IC 50 the best values were chosen. MTT colorimetric assays determined the cell viability at every time point. As shown in figure(3), the half-maximal inhibition concentration (IC 50) values of purified alginate treated WRL68 after 24,48,72 hours respectively of incubation at 37°C was 187.8, 62.0, 83.07 µg/ml and the IC 50 value of purified alginate treated HepG2 cells after 24,48,72 hours respectively of incubation at 37°C was 102.3, 42.5, 61.03 µg. The results showed that the IC50 of purified alginate treated A549 cells after 24,48,72 hours respectively of incubation at 37°C was 117.8, 57.06, 65.7 µg/ml and the IC50 of purified alginate treated WRL68 cells after 24,48,72 hours respectively of incubation at 37°C was 126.2, 63.57, 90.31 µg/ml shown in figure-4. Through a comparison of the half-maximal inhibition concentration (IC 50) between the Hep G2 cancer cell line and normal WRL68 cell line for three incubation times, the highest difference between IC50 concentrations was shown within 24 hours of incubation, while other times were the lowest difference between the inhibition concentrations of each incubation time. From these results, it was found that there clear effect of purified alginate on Hep G2 cells higher than normal WRL68 cells. The results showed clear effect of purified alginate on cells viability for these two cell lines and this effect increased with increase concentration. While, comparison of the half-maximal inhibition concentration (IC 50) between the A549 cancer cell line and normal WRL68 cell line for three incubation times, the highest difference between IC50 concentrations was shown within 72 hours of incubation. In general, cytotoxicity effective of Purified alginate( in vitro) was more effect on HepG2 cancer cells and less effect on A459 cancer cells with compared WRL68 normal cells.

![Figure (3): Cytotoxicity effect of purified alginate on HepG2 and WRL68 cells after 24 and 72 hrs of incubation at 37°C.](image)

Other study on supernatant fraction of secreted virulence factors from *P. aeruginosa*, at concentration 3.42 µg / ml, the supernatant cytotoxicity only showed significant impact on the A549 cells after 48h of incubation. In this case, the HB13 strain in all incubation periods was unable to achieve any significant cell death. Whereas, supernatant cytotoxicity at the 20.50 µg/ ml concentration demonstrated a significantly higher percentage of cell death compared to the prior concentration (3.42 µg / ml) (17). Also, three factors analyzed (copper oxide nanoparticles, alginate biopolymer, and stirring time) exhibited a significant effect in inhibiting cancer cell growth. The effects of factors including copper oxide nanoparticles, alginate biopolymer and stirring time on the inhibitory effect on the growth of MCF-7 cancer cells in the structure...
of alginate-copper oxide nanocomposite were 69.01%, 66.04% and 65.80%, respectively. Hence the synthesized nanocomposite can be used in the design of new anticancer drugs under optimal conditions\(^{(18)}\)

**Figure (4): Cytotoxicity effect of purified alginate on A549 and WRL68 cells after 24 and 72 hrs of incubation at 37°C**

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**Conclusion**

cytotoxicity effective of Purified alginate\( (\text{in vitro})\) was more effect on HepG2 cancer cells and less effect on A459 cancer cells with compared WRL68 normal cells.

**Conflict of Interest:** No conflict of interest

**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee

**References**


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Efficacy and Safety of Sacral Neuromodulation in Treatment of Refractory Overactive Bladder

Mohammed Bassil Ismail1, Wameedh Qays Abdullhussein2

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2Specialist CABMS (NEUROSURGERY), Medical City Complex, Baghdad

Abstract

Background: Overactive bladder syndrome is a urinary disorder that occurs more frequently in women and older people and is characterized by feeling an urge to urinate (sudden urge to urinate, with the feeling that you cannot get to the bathroom in time), doing it many times throughout the day (more than six) or having episodes of urinary incontinence (involuntary loss of urine) and nocturia (waking up several times during the night to urinate). In addition, other secondary symptoms may also occur, such as headache, dry mouth or blurred vision, among others. It is a problem that has a clear impact on the quality of life of those who suffer it and that can lead them to significantly reduce their social activity and negatively condition their work.

Overactive bladder (OAB) is defined as urinary urgency with or without urgency urinary incontinence, nocturia, and/or frequency in the absence of UTI or other obvious pathology. Sacral neuromodulation is a newer surgical therapy for refractory OAB.

Aim of Study: Is to assess the sacral neuromodulation (SN) safety and efficacy in refractory overactive bladder patients.

Patients and Methods: In the period from November 2015 to May 2017, the unit of neurogenic bladder and neuromodulation in (Gazi Al_Hariry) surgical specialty hospital, medical city complex 27 patient aged from (17—55) year old were complaining from refractory overactive bladder. All the 27 patients underwent stage1 & stage2 SNS devices implantation.

Results: Patients of urgency incontinence (group1) demonstrated that the number of leak was significantly reduced (p=0.01) after implantation of SN, (11.6_2.7) leak/day pre& post SN, numbers of pads (6.9 _ 0.8) pad/day pre&post SN. Patients with urgency frequency (group 2), numbers of voids/day pre & post SN implantation decreased significantly (p=0.01) (14.7 _ 6) voids /day. The voided volume increased significantly (p=0.02) from (136.4 _ 371.8) ml/void. The urgency episodes were decreased significantly (p=0.01) from (6.4 _ 2.1) episode/day. The complications occurred in 5 patients (18.5%). Two patients (7.4%) developed pain after trauma to the back, lead migration, lack of efficacy of device, treated by reprogramming the device, one patient (3.7%) get infection at the site of device implantation, SN was removed , there was one female patient (3.7%) got pregnancy and devise was deactivated. One patient (3.7%) complaining from pain and discomfort at the device site was treated conservatively.

Conclusion: Sacral neuromodulation is FDA approved as option for treatment of refractory OAB. There is expanding of utilizing of SN in the past two decades. According to multiple studies in addition to our study results. The sacral neuromodulation is safe and efficacious treatment option for refractory OAB.

Keyword: Neuromodulation, OAB, UTI, incontinence

Introduction

Overactive bladder syndrome is a urinary disorder that occurs more frequently in women and older people and is characterized by feeling an urge to urinate (sudden...
urge to urinate, with the feeling that you cannot get to the bathroom in time), doing it many times throughout the day (more than six) or having episodes of urinary incontinence (involuntary loss of urine) and nocturia (waking up several times during the night to urinate). In addition, other secondary symptoms may also occur, such as headache, dry mouth or blurred vision, among others. It is a problem that has a clear impact on the quality of life of those who suffer it and that can lead them to significantly reduce their social activity and negatively condition their work.

During the nineteenth of the 20th century the Overactive bladder was described as a group of bladder storage symptoms consisting of urgency, frequency, nocturia, and urinary incontinence. These symptoms without diagnosis due to detrusor overexertion, which was an objective urodynamic detection of involuntary detrusor activity. Overactive Bladder (OB) is defined as urgency of urination with incontinence or no seizures, night and/or frequency in the absence of IPS. Another obvious pathology. Wet OAB is defined as involuntary loss of urine associated with a strong desire to empty.3 Wet OABs have a huge gradient of severity and can have a significant negative impact on quality of life due to significant annoying symptoms and sudden loss of urinary control. (4)

OAB, specifically moist OAB, symptoms now disturb a significant rate of the population, and the overall prevalence of OAB has been shown to be comparable between men and women. A study conducted in the United States showed a prevalence of OAB in 16% of men and 16.9% of women. (4)

Milsom et al. in their study agree with that mentioned by the population of Europe, with an estimated prevalence of OAB (15.6%) in male and 17.4% in female on reviews finished by patients with in age older than 40 years. A correlation has been found between increasing age and prevalence of OAB wet symptoms. (5) Stewart et al found that the prevalence of wet HF symptoms increases with age for both men and women, and increases significantly after age 44 for women and age 64. For men. Stress and mixed urinary incontinence are also more common in the elderly, but patients with purely moist OAB are more likely to need treatment. (4)

The US Food and Drug Administration approved CH in September 1997 to treat nausea incontinence for those patients who were unsuccessful in managing their symptoms with more conservative therapies. They later approved HF for urinary retention syndrome and urgency. (6)

SN uses electrical stimulation to stimulate the bladder pacemaker, which is the sacral nerves that supply the pelvic floor and lower urinary tract muscles. Using electrical stimulation, you can inhibit or excite neural reflexes. (7)

**Aim of the Study:** Is to assess the sacral neuromodulation (SN) safety and efficacy in refractory overactive bladder patients.

**Patients and Methods**

In the period from November 2015 to May 2017, the unit of neurogenic bladder and neuromodulation at (Gazi Al_Harriry) surgical specialty hospital, medical city complex 27 patient aged from (17_55) year old complaining from refractory overactive bladder (Patients not responed or not tolerate conservative treatments for more than 3 months). All patients undergone full detailed history with physical examination with emphasis on the genitourinary and neurologic examinations, voiding diaries, urodynamic Studies, lumbosacral MRI, and flexible cystoscopy.

**Exclusion criteria:** -

1. Mechanical bladder outlet obstruction, bladder anatomical abnormalities.
2. Patient’s planning to undergo diathermy (shortwave; microwave; ultrasound) or MRI in the future.
3. Inadequate response during test stimulation intra op.
4. the patient unable to operate the device.
5. patient age less than 16 years.
7. Anatomical bony abnormalities of the sacrum, in which trans foraminal access could be difficult or impossible.
After that we divided these patients in two groups.

- Group1 (16 Patients with urgency incontinence.
- Group2) 11 Patients with urgency frequency.

All the 27 patients underwent stage1 & stage2 SNS devices implantation. After taking the informed consent from the patients.

Patients are taught keep a bladder diary during the trial stage (2weeks) to assess if there is a significant and worthwhile improvement in OAB symptoms.

If the test stage success in reducing the symptoms of bladder overactivity by at least (50%) in at least one of the following then we can proceed to permanent pulse generator implantation:

The advantage of the test stage is the ability to test out the SNS device over several days to see if it is suits the individual before committing to the treatment.

The patient and doctor decide together before the 2nd stage procedure if the SNS device has made enough of a difference to symptoms to proceed to the permanent pulse generator implantation.

All patients were reevaluated using voiding diary after two weeks, one month, three, six months and 1 year after the insertion of permanent SNS device and seeking for complication if found.

**Statistical Analysis**

SPSS version 23 was used for data entry and analysis. Mean and standard deviation were used to represent the numerical data while the frequency and percentage was used to represent the categorical data. Appropriate tests (chi square test, paired sample t test) were used to confirm significance where the significance level was set at \( p < 0.05 \).

**Results**

The results showed that the mean age of group with urgency incontinence was significantly lower than that of group with urgency frequency syndrome (26.6 ±3.6 sd, 36.7±2.8 sd) respectively as well as the results revealed that male patients represented 68.8% of first group and 81.8 of later group but the difference was considered non-significant as seen in table 1.

<table>
<thead>
<tr>
<th>Table.1: Sociodemographic characteristic of studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>urgency incontinence</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Age/year</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
</tbody>
</table>

The results of patients (group1) of urgency incontinence demonstrated that the number of leak was significantly reduced (\( p = 0.01 \)) after implantation of sacral neuromodulation as the mean value of number of leaks was 11.6/day pre implantation of the device and become 2.7 leak/day post implantation of the device with mean difference of 8.9 leaks/day. The same significant difference was reported with mean value of number of pad as it was 6.9 pads/day before implantation and become 0.8 pads/day after device implantation with mean difference of 6.1 pad/day as displaced in table 2.
Table 2: Mean difference for no. of leak, no. of pad pre and post sacral neuromodulation

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Mean difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of leaks /day pre</td>
<td>11.6</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of leaks /day post</td>
<td>2.7</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of pads/day pre</td>
<td>6.9</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of pads/day post</td>
<td>0.8</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The findings of current study of patients with urgency frequency (group 2) showed that the numbers of voids/day pre & post SN implantation was decreased significantly (p=0.01) from a mean of 14.7 voids/day to 6 voids/day with mean difference of 8.7 voids/day. The voided volume increased significantly (p=0.02) from a mean of 136.4 ml/void to 371.8 ml/void with mean difference of 235.4 ml/void of voided volume. The urgency episodes decreased significantly (p=0.01) from 6.4 episode/day prior to implantation to 2.1 episode/day after implantation of the device with mean difference of 4.2 urgency episodes/day as displaced in table 3.

Table 3: Mean difference in mean value of void, volume void and urgency episodes pre and post implantation of sacral neuromodulator device

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Mean difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of voids/day pre</td>
<td>14.7</td>
<td>2.9</td>
<td>8.7</td>
<td>0.01</td>
</tr>
<tr>
<td>No. of voids/day post</td>
<td>6.0</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>volume/void pre</td>
<td>136.4</td>
<td>30.7</td>
<td>-235.4</td>
<td>0.02</td>
</tr>
<tr>
<td>volume/void post</td>
<td>371.8</td>
<td>84.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urgency episodes pre</td>
<td>6.4</td>
<td>2.2</td>
<td>4.2</td>
<td>0.01</td>
</tr>
<tr>
<td>urgency episodes post</td>
<td>2.1</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Our data indicated that complications were reported with three cases of (group 1) with urgency incontinence. 1st one device was deactivated after pt. discovered pregnancy, 2nd one; patient developed pain & discomfort at device site that treated conservatively and 3rd one; patient got pain after trauma to the back, lead migration and lack of device efficacy treated by reprogramming the device.

While complication reported with two cases of (group 2) with urgency frequency, 1st case developed pain after trauma to the back, lead migration and lack of device efficacy treated by reprogramming the device, and 2nd case developed pain due to surgical site infection where device was removed. as seen in table 4.
Table 4: Incidence rate of complications with two groups

<table>
<thead>
<tr>
<th>Complications</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>urgency incontinence</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>device deactivated after pregnancy</td>
<td>1</td>
</tr>
<tr>
<td>pain &amp; discomfort at device site treated conservatively</td>
<td>1</td>
</tr>
<tr>
<td>Pain after trauma to the back, lead migration, reprogramming the device</td>
<td>1</td>
</tr>
<tr>
<td>Infection at the site of device implantation of device; SN was removed</td>
<td>0</td>
</tr>
<tr>
<td>Non</td>
<td>13</td>
</tr>
</tbody>
</table>

When we evaluate the percentage of complication in all patients of OAB included group1 and group 2 underwent SN implantation. We found the complications occurred in five patients (18.5%). Of these complication, 2 (7.4%) patients developed pain after trauma to the back, lead migration, lack of efficacy of device. Treated by reprogramming the device, one patient (3.7%) get infection at the site of device implantation, SN was removed, there was one female patient (3.7%) got pregnancy and devise was deactivated. And One patient (3.7%) complaining from pain and discomfort at the device site was treated conservatively.

Discussion

Many studies were carried to determine the effectiveness of sacral neuromodulation. Latini et al had seen its effects on 41 patients with urgency incontinence; and discovered that 90% of patients had a more than 50% improvement in signs and symptoms of urgency incontinence as noticed by voiding diaries and number of pads. (8)

The frequency of incontinent episodes for the patients in his study declined significantly from a mean of 8.8/day to 2.3/day for 6-month follow-up duration, numbers of pads were also reduced in patients undergone neuromodulator implantation, the mean number of patients’ diapers changed from 4.7 diapers per day to 0.8 diapers/day in follow-up period. (8)

Our results after 1 year of SN. implantation, (group1) patients with urgency incontinence demonstrated that the number of leak was significantly reduced (p=0.01) after implantation of sacral neuromodulation as the mean value of number of leaks was 11.6/day pre implantation of the device and become 2.7 leak/day post implantation of the device with mean difference of 8.9 leaks/day. The same significant difference was reported with mean value of number of pad, as it was 6.9 pads/day before implantation and become 0.8 pads/day after device implantation with mean difference of 6.1 pad/day.

Urgency & frequency symptoms get similar benefits for those with urgency incontinence. Charter_Kastler et al, in his study to evaluate the efficacy of SN for 9 patients with urinary frequency and found the voids/day decreased from (16.1 void/day to 8.2 void/day). And increase in max.bladder capacity from 244 mL to 377 mL and increase in the ability of patient to hold a greater volume between voids and over active bladder episodes (9)

Worldwide clinical study in seventeen centers van Kerrebroeck el la. 25 patient with urgency-frequency
underwent SN implantation, mean number of voids/day decreased significantly from 19.3 to 14.8, with (40%) clinically success after five years. Volume voided per void also increased (92.3 ml to 165.2 ml) with clinical success (56%) after five years. The clinical success rate in decreasing episodes of urgency (56%). An important finding in this study is the high correlation between success rates for treated patients for one and five year; indicating durable response with SN\(^{(10)}\)

Our study’s results of patients with urgency frequency (group 2) showed that the numbers of voids/day pre & post SN implantation decreased significantly (p=0.01) from a mean of 14.7 voids/day to 6 voids/day with mean difference of 8.7 voids/day. The volume voids increased significantly (p=0.02) from a mean of 136.4 ml/void to 371.8 ml/void with mean difference of 235.4 ml/void of voided volume. The urgency episodes were decreased significantly (p=0.01) from 6.4 episodes/day prior to implantation to 2.1 episodes/day after implantation of the device with mean difference of 4.2 urgency episodes/day

Hassouna et al. take of 51 patients post SN implantation from 12 centers underwent baseline assessment, including a detailed voiding diary, urodynamic evaluation that was showed improvements in cystometry study and life quality according Health Survey. It was shown that the effects were attributed to SN because of when SN were deactivated their patients symptoms returned to baseline. after reactivate the stimulator the benefits were returned again\(^{(11)}\)

There were numbers of complications that occurred in some patients who underwent SN implantation like any surgical intervention.

At a mean follow-up of ~3 years, White et al. followed up 221 patients for 3 years and found that 30.3% developed complication that required surgical intervention. These complications such as pain; infection; device malfunction; hematoma and lead migration. Of these complications (3.5% surgical site infection), (2.7% pain which was related to the device) and (5.9% lead migration). According this classification, they found the total complications rate (30.3%) and suspect that would represent the upper percentage end of complication\(^{(12)}\)

In our study; when we evaluate the percentage of complication in all patients of OAB included group1 and group 2 underwent SN implantation. We found the complications occurred in 5 patients (18.5%). Of these complication, One patient (3.7%) got infection at the site of device implantation, SN device was removed, And One patient (3.7%) complaining from pain and discomfort at the device site was treated conservatively by simple analgesia. Two patients (7.4%) developed pain after trauma to the back, lead migration, lack of efficacy, treated by reprogramming the device. Lead migration is usually resolved by reprogramming and usually does not require a new lead to be inserted. This can also define the problem at each electrode and is handled by reprogramming to move the circuit of stimulation away from the broken or migrated electrode\(^{(13)}\)

During follow up of our patient there was a female patient (3.7%) who got pregnancy. Device was deactivated to be activated after delivery. It is unclear whether SN is safe in pregnant patients or not. Also, it’s unclear whether it is teratogenic or not; especially in the first trimester. Wiseman et al. looked at six pregnant patients with SN that didn’t deactivated in the first trimester of pregnancy. This study shows that one of the six cases delivered a premature baby by 6 months but with no fetal abnormalities seen in any of these cases. This result promising that SN has no harmful effects to the pregnant women and her fetus. However, the manufacturers recommend that the pulse generator should be deactivated in female patient getting pregnancy\(^{(14)}\).

**Conclusion**

Sacral neuromodulation is FDA approved as option for treatment of refractory OAB. There is expanding of utilizing of SN in the past two decades.

According to multiple studies in addition to our study results, sacral neuromodulation is safe and efficacious treatment option for refractory OAB.

**Conflicts of Interest:** No

**Source of funding:** Self

**Ethical Clearance:** was taken from the scientific committee of the Iraqi Ministry of health
References


Retinoic Acid Treatment of Human Hematological Malignancies Induces Caspase Dependent and Independent Apoptotic Cell Death

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¹Lecturer/Al-Kufa Technical Institute, Al-Furat Al-Awsat Technical University, ²Assistant Professor/Biotechnology Department/College of Science/University of Baghdad/Iraq, ³Assistant Professor /Experimental Therapy Department, Iraqi Center for cancer and medical genetics research, Mustansiriyah University.

Abstract

The unprejudiced of this education is to gauge the ability of the retinoic acid to induce apoptotic cell death in hematological tumors through caspase dependent or independent apoptotic pathway. The cytotoxicity effects of retinoic acid of different concentrations (400,350,300,250,200,150,100,50,25,12.5 µg/ml) and exposure for all hematological malignancy cell lines (Human non-Hodgkin lymphoma SR and human multiple myeloma (COLO 677) and Human Monocytic Leukemia THP1 and Acute promyelocytic leukemia NB4) have been determined using a microtetrazolium (MTT) assay. Propodeum iodide and alcidine orange (AO/PI) paired discoloration was used to study the ability of retinoic acid to induce apoptosis in the infected cells and examined under fluorescence microscope and quantified for the percentage of apoptosis induction. Quantitative immunocytochemistry assay was used to study the caspase dependent and independent proteins expression in infected and control cells. Cells treated with Retinoic Acid showed increased cell death percentage compared to the untreated cells as quantified by MTT assay. AO/PI results revealed that Retinoic Acid had powerful effect on inducing apoptosis significantly (p<0.001) in human cancer cell lines tested, compared to control cell. Immunocytochemistry in Retinoic Acid infected human hematological cell lines revealed remarkable increase in expression of caspase 8,9 (dependent pathway) and AIF, ENDOG (independent pathway) induces a significant (p<0.002) as compared untreated cell.

This study, which shows the role of the Retinoic Acid in inducing apoptosis through a dependent and independent pathway in cancer cells, we anticipation these annotations will shanty light on the impending exploration of retinoic acid in cancer hindrance and rehabilitation.

Keywords: Retinoic Acid, SR, NB4, THP1, COLO677, Apoptosis

Introduction

A trademark variation from the norm of leukemia cells is that they are hindered at a beginning period of their advancement and neglect to separate into useful develop cells. During the 1970s and 1980s, a few logical accomplishments promoted the procedure of actuating harmful cells to defeat their square of separation and enter the apoptotic pathways as an exquisite option in contrast to murdering disease cells by cytotoxic therapies[1]. This mediation could hypothetically confine introduction to undesirable reactions of cytotoxic chemotherapy, and all the more significantly, improve total reduction and fix rates, endeavors to clarify substances to control the separation of myeloid leukemia,2 and the principal proof of the separating properties of retinoic acid[2, 3]. The potential for separating treatment to improve fix rates in leukemia is exemplified by the advancement of all-trans retinoic corrosive for the focused on treatment
of intense promyelocytic leukemia. One of the most amazing aftereffects of starting in vitro analyses was accomplished in separating HL-60 cells per ATRA, which bent terminal separation in 90% of cells per 10−6 M retinoic acid\[2\].

Retinoids are a class of normally happening aggravates that are fundamentally identified with nutrient An (or retinol). Retinoids control a wide scope of organic procedures, including improvement, separation, multiplication, and apoptosis \[4\]. ATRA is the dynamic metabolite of nutrient An and intercedes its organic impacts by enacting at least one of the firmly correlated retinoic corrosive receptorse (RARα, RARβ, and RARγ) that aptitude as ligand-subordinate transcriptional controllers. These receptors structure heterodimers with rexinoid receptors (RXRα, β, and γ) and tie to retinoid responsive reaction components situated in the advertiser district of retinoid target qualities to animate quality translation \[4\].

A functioning common retinoid, all-trans retinoic corrosive, is viable in separation treatment for intense promyelocytic leukemia \[5\], is a subtype of intense myeloid leukemia, which is portrayed by a particular chromosomal variation from the norm t(15,17) related with a hereditary revision between retinoic corrosive receptor α (RARα) (quality image, RARA) and the promyelocytic leukemia quality PML\[6\]. RARα assumes a job in granulocytic separation of hematopoietic cells and the irregular illusory receptor PML-RARα has been involved in APL pathogenesis by obstructing the myeloid separation program and upgrading self-reestablishment of leukemic cells\[7, 8\]. Pharmacological portions of ATRA instigate separation of APL cells into granulocytes through corruption of PML-RARα and recuperation of physiological RARα flagging \[7\].

Nutrient An is particularly significant in light of the fact that it can’t be combined by creatures and must be provided from an eating regimen that incorporates plants \[9\], suppress malignant transformation in vitro \[10\]. Sure of these mixes restrain the development of changed cells \[11\] and actuate separation of mouse embryonal carcinoma cells in vitro \[12\]. The antineoplastic impacts of retinoids recommend that these mixes could be utilized restoratively for the chemoprevention of malignancy \[2\]. ATRA actuates terminal separation of leukaemic cell lines, so ATRA-based treatment can prompt total reduction in APL \[13\]. Tumor improvement, angiogenesis and metastasis are the organic capacities hindered by retinoic acid \[14\]. It has likewise been found that retinoic corrosive directs mitochondrial porosity, passing receptors, ubiquitination, and types of responsive oxygen, etc.\[15\]. The inhibitory effects of retinoic corrosive are believed to be cultivated by initiating the receptor of retinoic corrosive (RAR) or the receptor of retinoic X. rar and rxr structure ligand restricting heterodimers and aptitude, o change downstream quality articulation, rar and rxr travel into cell cores and tie to the retinoic corrosive response components (Uncommon) set in the 5’ downstream qualities of retinoic corrosive\[16, 17\]. Actuating the old style pathway above will bring about cell separation, capture and eventually apoptosis \[16\]. The pathways on that RA demonstrations need adjusted convergences of this retinoid, and distortion on the degree of retinoic corrosive from ordinary outcomes in irregular development and advancement \[18\]. The activity of retinoic corrosive relies upon different procedures, just as combination and degeneration of RA, phosphorylation and debasement of RA receptors, and enrolling of chromatin remodelers and procedures worried inside the vehicle, spread, and cell ingestion of retinoids \[19\], notwithstanding the previously mentioned great pathway, retinoic corrosive can likewise control downstream quality articulation by adjusting other interpretation factors, for example, NF-b, IFN-J, TGF-b, MAPK, and even chromatin rebuilding \[20\]. RARs/ RXRs heterodimerize and control the motioning of these accomplice receptors, including non-exemplary or non-genomic forms, These accomplice receptors some of the time have opposite capacities to RARs/RXRs, including retinoid flagging and digestion, cell bond, cell-grid collaboration, and cytoskeleton renovating in their appearance microarray assessment\[21\].

**Materials and Methods**

**Preparation of cells and cell culture**

This investigation remained affirmed via Baghdad College, School of Science, biotechnology office. The human cell line non-Hodgkin lymphoma SR (CD20 –) was generously given by Dr S.J. Russell, Mayo Center, Atomic Drug Office (Rochester, M N, USA) and refined in altered Falcon’s media (US Organic, Salem, Mama,
USA) with 5% fetal ox-like serum (Capricorn Logical).

The human various myeloma (COLO 677) was initially portrayed as being gotten from a tumor in the left axillary lymph hub of a 39-years-old male with little lung cell carcinoma in 1989. Be that as it may, DNA fingerprinting recommends cross-sullying with cell line RPMI-8226; RPMI-8226 was built up from the fringe blood of a 61-year-old male with various myeloma in 1966. The THP1 (Human monocytic leukemia) Got from the fringe blood of a 1-year-old male with intense monocytic leukemia.[22]

The NB4 cell line Intense promyelocytic leukemia (APL) is a well-characterized substance among intense leukemia, cytogenetically described by a t(15;17) (q22;q11-12) translocation [23], was acquired from the Division of Test Treatment, Iraqi Community for Disease and Restorative Hereditary Exploration (ICCMGR; Baghdad, Iraq). Every one of the cells were refined in Roswell Park Commemoration Establishment 1640 medium (US Natural) with fetal calf serum 10%, penicillin 100 u/ml, and streptomycin 100 μg/ml, and brooded at 37°C.

Retinoic Acid

In this study Retinoic acid (RA) was used as a chemotherapeutic agent by dissolving 7 mg of RA powder in 5 ml (1%DMSO and complete free serum medium RPMI and filtered by syringe filter 0.2 μm to prepare stock solution 5mM. Then diluted with 3 ml of serum free medium until before using for in vitro studies[24]

Cytotoxicity Assay

The cells remained seeded in 96well saucers and were eroded with phosphate cradled saline before vaccinating with and without the Retinoic Corrosive at various focus (400,350,300,250,200,150,100,50,25,12.5). Following 72 hours brooding, the intermediate was suctioned and an absolute of100 µl of MTT arrangement (5 mg/ml in PBS, pH 7.2) remained supplementary to separately well and the plates were hatched for 2 hours at 37 °C. After hatching, 50 µl of dimethyl sulfoxide was supplementary to each well, trailed by delicate shaking for 45 min to dissolve the color of formosan. The receptiveness was resolved on a microplate peruser at 584 nm wavelength. The examine was achieved in triplicate for each of the ailment[25], The repressing pace of cell development (the cytotoxicity level was determined as (IG%) = (a-b)/ax100, where a is the mean optical thickness of untreated wells and b is the optical thickness of treated wells [26]

Apoptosis quantification using acridine orange and propidium iodide double staining:

Acridine orange is an interposing color that can infiltrate equally animate and dead cells. AO will recolor every single nucleated cell to create green fluorescence.

Propidium iodide can just arrive departed cells with poor layer honesty, so it will recolor all dead nucleated cells to produce red fluorescence. Cells recolored with both AO and PI fluoresce red because of extinguishing, so all live nucleated cells fluoresce green and all dead nucleated cells fluoresce red. 1μl Acridine orange stock (5mg/ml) and 1 μl propidium iodid stock (3mg/ml) blending with 1 ml PBS (phosphate cushion arrangement). We included AO/PI to the tried wells after media expulsion from each well and including 50 μl of blend arrangement. Following 20 second we expelled the stain from the well, and quickly saw under fluorescent microscope[27]

Immunocytochemistry

For the disciple cell lines (NB4 and SR) were refined on coverslipes. The cells were permitted to build up a one layer. After this introduction of RA (IC50), after 72hrs. At that point, the cells were fixed with cold CH3)2CO at 2 – 5 minutes, and then expelled cold CH3)2CO and eroded with phosphate-cushioned saline for multiple times and late to dry. Then slides were hatched in a humidified chamber for 10 minutes with H2O2 1% after obsession, eroded a few times with PBS, and brooded with 1.5% blocking reagent for thirty to forty minutes at room temperature. At that point, the essential immune response of the CAS-8, CAS-9, AIF and ENDO G counter acting agent for 1 - 1:30 hrs., eroded a few times with PBS, A while later, include auxiliary immune response and remain for two hrs. It was recolor by the ImmunoCruz™ mouse ABC recoloring framework. Later counterstained with Hematoxylin for thirty to sixty seconds after eroded widely with PBS, and. The slides were mounted with Distyrene, a plasticizer, and xylene, investigated utilizing light microscopy, and
Results

Cytotoxicity of Retinoic Acid:

In this examination, the cytolytic impacts of Retinoic Corrosive on hematological danger cell lines, ordinary HBL – 100 cell line, were dictated by estimating the cytotoxic portion that execute half of the cell populace when contrasted with the untreated control for 72 hr. utilizing colorimetric cytotoxicity test (MTT). The examine was rehashed multiple periods. The level of feasible cells was conspired against RA and the various fixations (400, 350, 300, 250, 200, 150, 100, 50, 25, 12.5 µg/ml). qualities were resolved after 72 hr. RA indicated cytotoxicity impact on lymphoma SR cell line and human different myeloma (COLO 677) and Human monocytic leukemia (THP1) and Intense promyelocytic leukemia (NB4) cell lines and HBL-100 ordinary cell line in portion subordinate way. The outcome was a noteworthy (p<0.0001) increment in RA cell passing and cytotoxicity sway on hematological threat and low cytotoxic on HBL-100 typical cell line. (Figure 1, A, B, C, D and E)

A: Showing the RA’s cytotoxicity impact on the cell line COLO677
B: Showing the RA cytotoxicity impact on NB4 cell line

C: Showing the RA cytotoxicity impact on the SR cell line
D: cytotoxicity effect of the RA on THP1 cell line

E: Cytotoxicity effect of the RA on HBL-100 normal cell line.

Figure 1: Cytotoxicity effect of the Retinoic Acid on Five different cell lines, represented by (A, B, C, D, and E).
Quantification of apoptosis using propidium iodide and acridine orange double staining:

This examination was done to recognize the changes in morphology and the extents of apoptotic, necrotic and typical feasible cells in the number of inhabitants in SR and NB4 discilpe cell lines presented to RA at IC50 for 72 h contrasted with untreated cells. AO will recolor every single nucleated cell to create green fluorescence. Propidium iodide can just arrive departed cells with pitiable film integrity, so it will recolor all dead nucleated cells to produce red fluorescence. Cells recolored per equally AO and PI fluoresce red because of extinguishing, so all live nucleated cells fluoresce green and all dead nucleated cells fluoresce red. The percentage of viable SR cells unprocessed with RA gave 76.615% at 72 h but the percentage of apoptotic cells had significantly (p < 0.001) and gave 117.519%, at 72 h. On the other hand, The percentage of viable NB4 cells untreated with RA gave 75.105% at 72 h but the apoptotic cells percentage had significantly (p < 0.0001) and gave 105.62% at 72 h. as shown in (Figure 2).

Figure 2: The apoptotic by use acridine orang stain: (A) control viable green cells SR cell line, (B) cell infected with RA red cell SR cell line, (C) control viable green cells NB4 cell line, (B) cell infected with RA red cell NB4 cell line
Immunocytochemistry

Immunocytochemistry results showed that NB4 and SR cell lines were sure for ward pathway CAS-8 and CAS-9 and positive for free pathway AIF and Endonuclease G this test was done to affirm that focused treatment utilizing Retinoic Corrosive is compelling and the focused on antigen is available. The outcomes exhibited that the action of caspase-8 was altogether (P>0.0001) and caspase - 9 was essentially (P>0.001) (expanded in both cell lines treated with RA (Figure 3).

This affirmed by utilizing explicit monoclonal antibodies for caspase 8, 9 (ward) and AIF and Endonuclease G proteins for apoptosis. lymphoma SR cell line, Intense promyelocytic leukemia NB4 cell line were recolored positive for articulation of caspase 8,9, AIF and Endonuclease G proteins in the tumor cells, This test was done to distinguish RA prompting apoptosis component in various cells in vitro after 72 hr. from contamination, (Antigens in tainted cells stains dark colored). Uninfected cells do not stain. (Nuclei stain blue by counter stain).

Figure 3: Immunocytochemical study of lymphoma SR cell line (A1) control, (A2) CAS-8 antibody, (A3) Analysis, (B1) control, (B2) CAS-9 antibody, (B3) analysis, (C1) Control, (C2) AIF antibody, (C3) Analysis, (D1) Control, (D2) EndoG antibody, (D3) Analysis, (DAB stain) Magnification: 20×.
Discussion

The aim of this study was to investigate the involvement of caspase dependent or independent way of apoptosis through infection by Retinoic Acid.

In this investigation, we have assessed the potential antitumor impacts of the RA in leukemic cell lines. Our information demonstrate that RA applies antiproliferative and proapoptotic or separation initiating impacts in hematological cell lines. High convergences of RA gave low cytotoxicity practically identical with low Focuses, which was trailed by cell passing following 72 hours. Conversely, low convergences of RA instigated apoptosis. These information demonstrated that RA applies portion subordinate proapoptotic and antiproliferative impacts in leukemic cells. Moreover, a factually huge lower cell executing of typical HBL-100, It is discovered that, RA have been explored widely for their utilization in strong tumor disease counteractive action and treatment[29].

Retinoic destructive may be gained from either through the consistently use of plants in a reasonable eating regimen or through supplement upgrades. Under common conditions in the body, retinoic destructive does preventive kill threatening development course of action. After dangerous development course of action, retinoic destructive transforms into an attacker to illness cells, one that discourages their advancement and division moreover triggers their partition and going through express pathways[30].

In general, RA causes a square in the cell cycle stage G1, with an expansion in the extent of cells in the stage G0/G1 and an abatement in the extent of cells in the stage S [31]. RARβ2 is inducible to RA and is the prevalent receptor that intercedes the inhibitory effects of RA on cell proliferation [32, 33].

In this study, we showed that RA repress the development and actuate the apoptosis of RA-safe hematologic tumor, gave that they are additionally fit for initiating the caspase ward or caspase free pathway. The apoptotic cells concoction and morphological changes can be separated from any other cell demise through numerous strategies. In this investigation, affirmation of apoptosis started by RA was finished utilizing the minute assessment. AO/PI recoloring affirms that RA in cell societies instigates cell passing by means of the apoptotic pathway, the quantity of apoptotic cells uncovered that in treated hematologic cell lines the level of apoptotic cells relatively expanded with post-immunization time. Identification of the acridin orang and propidium iodid strategy (AO/PI) gives an early sign of the commencement of cell apoptosis[27].

RA is viable at the phone multiplication and separation just as the counter malignant growth capacities during the procedure of carcinogenesis (Zhu and Luo, 2016). RA have various receptors RAR, RXR and RARγ act with various pathways as Wnt/β-catenin and β-catenin/TCF, the RAR and RXR receptors restrain Wnt/β-catenin, while RARγ receptor goes about as a tumor oncogene lead to the actuation of Wnt/β-catenin pathway [34], these methods the RA demonstrations in two inverse ways, relied upon its receptors. While, RA repress malignancy cell expansion [34].

Retinoic acid likewise initiates the NF-κB endurance pathway [35, 36], ATRA prompts the outflow of cIAP2 and TRAF1 within the sight of elevated levels of cIAP1 and TRAF2 mRNAs, The attending articulation of these variables is thought to build up a complex at the TNF-R1 that prompts actuation of NF-κB by means of the NIK-IKK-IκB flagging course with the outcome that TNF-α, rather than inciting apoptosis, sets up a NF-κB–interceded autocatalytic endurance loop[37].

Quantitative picture examination for the immunocytochemistry test uncovered expanding in articulation of caspase-8 and caspase-9 (caspase subordinate pathway) in SR and NB4 cell lines. Caspase-8 was distinguished as an initiator caspase activated by death receptors. Along these lines, the initiation of caspase-8 recommended that RA may instigate apoptosis through the outward demise receptor-pathway. cells was seen in the immunocytochemistry measures, as contrasted and untreated cell Examinations of the component of RA-prompted cell passing demonstrated mitochondrial depolarization, translocation of phosphatidylserine to the cell surface, enactment of caspases, and DNA discontinuity, proposing apoptotic cell demise. To explicitly inspect the job of caspase-8,9 in RA-prompted apoptosis[38]. Late investigations have demonstrated that RA instigated Ca2 discharge from the endoplasmic reticulum in skin, bosom, and lung...
malignancy cells, and that Ca2 chelation hindered the apoptogenic impacts of CDDO [38]. Strangely, Ca2 can trigger AIF discharge from the mitochondria [39], which could conceivably provide a link between endoplasmic reticulum stress and caspase-independent cell death.

Notwithstanding the counter apoptosis exercises of BCL2A1 and NFκB, retinoic corrosive invigorates the articulation of the apoptosis inhibitors cIAP2 as well as of NAIP. Overexpression of IAPs restrains apoptosis instigated by master apoptotic BCL-2 relatives through their immediate official to the effectors casp-3 and casp-7, the initiator casp-9, and its expert enzyme19. Additionally, the retinoic-corrosive induced39 articulation of casp-8, which transfers TRAIL activity in the Plate, may encourage TRAIL–interceded destruction of leukemia blasts[40].

In conclusion, our study suggests that the Retinoic Acid is a very promising anti-hematological malignancy agent that found to act by inducing apoptosis through caspase dependent and caspase independent pathways of apoptosis.

Acknowledgment: The authors would like to thank the staff of experimental therapy department – Iraqi Center for Cancer and medical Genetic Research, Mustansiriya University for their support during the work.

Conflicts of Interest: Nil.

Ethical Clearance: Nil.

Funding: This study was self-funded.

References


Non-Hodgkin’s Lymphoma in the Parotid Gland Similar to Benign Lymphoepithelial Lesion: A Case Indonesian Male

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Abstract

Background: Primary parotid non-Hodgkin’s lymphoma (NHL) is a very rare case. Case presentation: A 66-year-old Indonesian male with a complaint of a lump in front of the right ear about 1 year with a history of postoperative primary parotid NHL 3 years ago. Patients underwent Fine Needle Aspiration Biopsy (FNAB) examination, showing a distribution of lymphoid cells with a diverse population (benign lymphoepithelial lesion / BLL) different from the results of FNAB 3 years ago. The patient underwent a right superficial parotidectomy, and the results of the histopathological examination revealed mature lymphocyte cells mixed with histiocytes, forming foci of the follicle in part with an active germinal center. Conclusion: NHL reports are necessary to minimize misdiagnosis between primary parotid NHL and BLL.

Keywords: non-Hodgkin’s lymphoma, benign lymphoepithelial lesion, parotidectomy

Introduction

Non-Hodgkin lymphoma (NHL) is found in 86% of all lymphoma cases, with a rough estimation of 90% covering B cell lymphoma. About 2-5% of primary NHL occurs in the head and neck region, one of which is in the parotid gland. Parotid gland NHL cases are very rare, limited to small and closed case series reports. Primary parotid gland NHL accounts for only 0.5-0.87% of all NHL cases, with increasing prevalence in the last few decades [1,2].

The diagnosis of primary parotid gland NHL in patients is difficult because of the extremely rare number of cases. In NHL case, lymphatic nodules are generally biopsied, whereas computed tomography (CT scan) / magnetic resonance imaging (MRI) only infoms about the origin of the malignancy and extraparotid expansion. To date, there have been no pathognomonic findings that indicate lymphoma on CT or MRI scans [3-5]. The purpose of reporting this case is to inform that there are similarities between the diagnosis of primary parotid gland NHL and benign lymphoepithelial lesion (BLL).

Case Presentation

A 66-years-old Indonesian male with complaints of a lump in front of the right ear about 1 year. The lump is enlarged slowly. The patient had surgery for the right parotid gland tumor 3 years ago. The results of the Fine Needle Aspiration Biopsy (FNAB) at that time showed the distribution of lymphoid cells of various maturity with some forming germinal centers. Visible groups of salivary gland epithelial cells also showed BLL.

The results of histopathological examination of postoperative tissue showed pieces of tissue consisting of dense proliferating lymphoid cells, small to medium sized, monotonous, smooth chromatin, diffused narrow cytoplasm, infiltrative between salivary glandular tissues. There were still follicles with germinal centers among them. Mitosis was difficult to find, and there

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was a normal salivary gland remained at the edge. A low-grade lymphoma was found in accordance with the description of lymphoepithelial sialadenitis (Mikulicz disease). Advice from anatomic pathology to ensure immunohistochemical examination in this preparation. Immunohistochemical results showed positive CD20 in lymphocyte cells surrounding the epimyoepithelial island, positive CD3 in T cells between follicles, and Ki67 was limited to germinal center. In accordance with the description of benign lymphoepithelial lesion (Mikulicz disease).

Physical examination found no abnormalities in ear, nose, and throat, as well as no cervical lymph node enlargement. The parotid gland had a mobile mass with a size of about 3x2x2 cm, dense and springy, flat surface, no tenderness. Intact right facial nerve (House-Brackmann scale grade I). Physical examination of axillary and inguinal glands did not reveal a mass (Fig. 1). Laboratory test results were normal, serological hepatitis B virus and negative hepatitis C virus.

FNAB showed smears with diverse lymphoid cells distribution. Unclear epithelial cells; showed lymphoid hyperplasia. Tumor excision was recommended for histopathological and immunohistochemical confirmation if necessary, because it had not been able to get rid of low-grade lymphoid neoplasms. MRI examination with contrast obtained a solid mass of size 5.4x1.7x3.9cm; indistinct border of irregular edge in the right parotid region. Contrasting obtained heterogenous contrast enhancement. The lesion appeared attached to the right masseter muscle. The conclusion supported the picture of benign parotid mass of the right muscle (Fig. 2).

Based on available data, a BLL type of parotid tumor was established. A week after the diagnosis, a right superficial parotidectomy surgery was performed with Blair’s modified incision. The skin incision was deepened into the subcutaneous tissue and platelet musculature to identify and preserve the right facial nerve branches, then proceeded with tumor removal (Fig. 3). The size of the tumor obtained was about 40 cm². The tumor tissue was sent to the anatomic pathology installation for histopathological examination (Fig. 4).

The results of post-operative histopathological examination showed extensive infiltration of lymphocytic cells surrounding the remaining glands and ducts that were pinched to form epimyoepithelial nests. Lymphoid tissue consisted of mature lymphocyte cells mixed with histiocytes, forming foci of the follicle with some active germinal centres. This description supported Mikulicz disease or lymphoepithelial sialadenitis, concluded Lymphoepithelial sialadenitis/BLL (Mikulicz disease).

Furthermore, the patient was recommended to have immunohistochemical examination. The results showed negative CD3 in tumor cells, positive in mature lymphocyte cells, positive CD20 in diffuse and condense on tumor cell membranes, and Ki67 etched 505 in tumor nuclei, immunoarchitecture supported non-Hodgkin lymphoma B cell type which could come from MALT lymphoma.

The results of the data obtained concluded that the patient was diagnosed having a right parotid NHL, postoperatively obtained right facial fascia (House-Brackmann scale II-III) which was subsequently recovered with physiotherapy, especially the submandibular marginal branches and right buccal. Subsequent therapy included chemotherapy with a combination regimen according to NHL for 6 cycles. Control in the second month after surgery showed that the function of right facial fascia nerve had begun to recover, although it still looked a little paresis (House-Brackmann scale grade II), especially the right lip corner. Control in the fifth month after surgery did not show any residual mass on the right parotid, the right facial nerve function was visible again (House-Brackmann scale grade I).
Figure 1. Physical examination shows a mass in the right parotid gland

Figure 2. MRI with contrast shows indistinct solid borderline lesions, irregular margins in right parotid region
Discussions

In this case, a CT scan was not performed but an MRI examination was immediately performed. A solid border lesion was found with an irregular border in the right parotid region. A heterogeneous contrast enhancement appeared; the lesion was attached to the right masseter muscle. The radiological features of parotid gland NHL were rarely well described. According to Corr et al., who presented a cohort study of 10 HIV-infected children with parotid gland NHL, a CT scan of the lesion contained a muted hypoechoic solid nodule, which was similar to hyperplastic lymphoid tissue or lymphoma. These lesions are cystic (from compression of the parotid duct terminal with adjacent hypoplastic or neoplastic lymphoid tissue) and separate with calcification, both intracystic and parenchymal can coexist. These radiological features have been described in the BLL found in patients with AIDS or Sjögren’s syndrome [4,6-8].

An FNAB examination had been conducted twice, with first examination showing a Mikulicz disease. The second test was carried out three years ago because a lump appeared again in the same place, and the conclusion was a lymphoid hyperplasia. The results of this FNAB indicated the origin of a malignancy that leads to NHL, as this finding is consistent with the literature. Preoperative FNAB from a suspected tumor and radiological examination in certain cases is part of the preoperative examination of parotid gland lesions. Parotid gland NHL is difficult to diagnose through FNAB cytology. FNAB examination for head and neck region NHL is the subject with the highest error rate of all FNABs in the head and neck, with a false-negative rate of 32%. Although FNAB can definitively diagnose...
lymphoma in some patients, tissue biopsy has a much higher accuracy \[4,6,7,9\].

A parotid surgery was conducted with a superficial parotidectomy approach with the aim of the diagnostic process and the therapeutic process. Patients with localized parotid gland tumors, in general, is highly recommended for surgical or surgical management to diagnose parotid gland NHL because histological evaluation is very important to treat NHL. Parotid surgery is recommended for therapy as well as to confirm histological diagnosis and further follow-up planning. The prognosis of patients with parotid gland NHL so far will be good if diagnosed immediately. Limited data shows that 80% of patients have a five-year survival rate \[3,4,6,7\].

This case, after being diagnosed as NHL type MALT parotid gland, immediately performed chemotherapy with NHL-compliant regimens. The patient was followed during chemotherapy and no residual mass was found after control for 6 months, showing an excellent therapeutic response. Diagnosis of NHL must involve an in-depth evaluation of the involvement and spread of the same disease must be carried out in other locations before starting local therapy. Radiotherapy and chemotherapy must be considered after surgery to eradicate spread or residue. Irradiation in cases of lesions localized in the early stages and chemotherapy in the advanced disease stage. Isobe et al., treated 37 patients with stage 1E extragastric MALT LNH with only radiotherapy, and around 97.3% of patients received an increase in the number of free survival rates over a three-year period of 91.9%. So far chemotherapy in patients with NHL is still a very sensitive therapy using the CHOP protocol (cyclophosphamide, doxorubicin, vincristine, and prednisone) \[2,5,10\].

This case has no lymph nodes or involvement of other organs, but only limited to the history and physical examination of the axillary and inguinal glands. This indicates that this NHL case is not a systemic type and is limited to the right parotid gland or primary to the parotid gland. Salivary gland NHL tends to be more aggressive and can occur in regional or distant lymph nodes in other organs. According to Wenzel et al., therapy with local surgery of patients with head and neck NHL has a relatively high risk of experiencing initial spread or recurrence \[2,5,10\].

**Conclusions**

A very rare and suspicious parotid NHL case has been reported as a transformation from BLL or Mikulicz disease. Primary and secondary NHL in the parotid gland are clinically indistinguishable from benign tumors or other malignant lesions. The imaging modality and cytology of FNAB does not always help, so the majority of patients require parotidectomy for diagnosis and therapy.

**Ethics Statement:** The present case report adhered to the Declaration of Helsinki. Informed consent for publication was obtained from the patient.

**Conflict of Interest:** The authors report no conflict of interest in this publish.

**Author’s Contribution:** All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

**Funding:** None

**References**


The Influence of the GETAR Spiritual Approach on Smoking Behavior of Junior High School Students in Palu City, Indonesia

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Abstract

Smoking cases at the age of children less than 18 years will have a very fatal impact because considering the age of the child who is still growing. GETAR (Movement without Smoking) spiritual approach uses the concept of religion, which in this case is exemplified by Islam by helping individuals to find their nature. The purpose of this study is to analyze the effect of the GETAR spiritual approach on smoking behavior of junior high school students in Palu City, Indonesia. This type of research is a quasy experiment. The sample consisted of 35 smokers in grade 7, 8, and 9 at SMPN 1 Palu as an intervention group, with saturated sampling technique, and 35 students in grade 7, 8, and 9 at SMPN 4 Palu. The data were analysis with paired sample t test and independent sample t test. This study showed that there are differences in knowledge (p = 0.000), attitudes (p = 0.000), and actions (p = 0.000) students of SMPN 1 Palu about smoking before and after GETAR spiritual intervention. And there are differences in the knowledge (p = 0.000), attitudes (p = 0.020), and actions (p = 0.000) between students who were given GETAR spiritual intervention (students of SMPN 1 Palu) and those who were not given GETAR spiritual intervention (students of SMPN 4 Palu). It concludes that there is a difference in the knowledge, attitudes, and actions of SMPN 1 Palu students about smoking before and after the spiritual intervention of GETAR. And there is a difference in the knowledge, attitudes, and actions of students between those who were given the spiritual intervention of GETAR (students of SMPN 1 Palu) and those who were not given the spiritual intervention of GETAR (students of SMPN 4 Palu).

Keywords: GETAR, Spiritual, Smoking, Behavior

Introduction

Smoking cases at the age of children less than 18 years will have a very fatal impact because considering the age of the child who is still growing. In addition to having an impact on health, it will also have an impact on the child’s future, because children have a strategic position in the life of the community and nation. There is no reason to assume that only those who deviate have the urge to do so, even people who have a good understanding of religion sometimes have the urge to behave deviantly, especially those who have less understanding of religion.

Interventions at the age of children tend to be more effective in preventing children from smoking when compared to adults who, if addicted to smoking, are very difficult to stop. Smoking, from ancient times until now for students is a serious offense. Anyone caught...
smoking, be prepared to receive punishment. As the rules for smoking prohibition in school environments get tighter, it turns out that the number of child smokers continues to grow.4

A spiritual approach is a solid foundation to build future generations of excellence. The advantage of GETAR spiritual intervention is that students are provided with assistance and provided with various educations about the verses of the Al-Quran and Hadiths about the prohibition and dangers of smoking and its impact on negative behavior and health. Suitable for children because they are still in their growing age and can form a good perception as early as possible about the dangers of smoking and non-smoking behavior through the presentation of interesting intervention materials, accompanied by interesting language and pictures. The substance of GETAR spiritual approach is material about the history of tobacco, various ways of smoking, smoking motivation, various kinds of diseases caused by smoking, children and the dangers of smoking, Islamic attitudes towards smoking, the benefits of leaving cigarettes, and how to avoid smoking.

WHO (World Health Organization) data in 2015 shows that the prevalence of active smokers in the world currently reaches 17.4%. The National Basic Health Research (Riskesdas) data in 2018 shows that the prevalence of Indonesian smokers in the population aged 10-18 years continues to increase, namely in the Riskesdas data in 2013 of 7.2%, 2016 National Circular Survey data of 8.8% and in the data Riskesdas in 2018 increased to 9.1%. The data presented shows that there is an increase in the prevalence of smokers aged ≥ 10 years in Central Sulawesi, by 30.7% with the number of smokers every day at 26.2% and smokers sometimes at 4.5%. Data related to age starting smoking every day for ages 10-14 years, the city of Palu ranks third largest in Central Sulawesi at 13.5%.

Seeing the problem above, the researcher was interested in conducting research on is there an effect of GETAR spiritual approach on the smoking behavior of junior high school students in Palu City, Indonesia. The purpose of this systematic review is to analyze the effect of the GETAR spiritual approach on smoking behavior of junior high school students in Palu City, Indonesia.

MATERIALS AND METHODS

This type of research is a quasy experiment. The sample consisted of 35 smokers in grade 7, 8, and 9 at SMPN 1 Palu as an intervention group, with saturated sampling technique, as a control group, and 35 students in grade 7, 8, and 9 at SMPN 4 Palu. The data were processed using univariate and bivariate analysis with paired sample t test and independent sample t test.

Findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th></th>
<th>After</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Knowledge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Good</td>
<td>8</td>
<td>22.9</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Good</td>
<td>27</td>
<td>77.1</td>
<td>30</td>
<td>85.7</td>
</tr>
<tr>
<td>Attitude</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Good</td>
<td>14</td>
<td>40.0</td>
<td>10</td>
<td>28.6</td>
</tr>
<tr>
<td>Good</td>
<td>21</td>
<td>60.0</td>
<td>25</td>
<td>71.4</td>
</tr>
<tr>
<td>Action</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td>35</td>
<td>100.0</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Do Not Some</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>91.4</td>
</tr>
</tbody>
</table>
Based on Table 1, these results indicate that there is an increase in good knowledge after the intervention of 3 respondents, namely 8.6%. Besides, these results indicate that there is an increase in good knowledge after the intervention of 4 respondents, namely 11.4%. Also, these results indicate that there is an increase in the act of not smoking after the intervention by 32 respondents, namely 91.04%.

**Table 2: Respondents Behavior Before and After GETAR Spiritual Intervention at SMPN 4 Palu**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Knowledge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Good</td>
<td>16</td>
<td>45.7</td>
</tr>
<tr>
<td>Good</td>
<td>19</td>
<td>54.3</td>
</tr>
<tr>
<td>Attitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Good</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Good</td>
<td>18</td>
<td>51.4</td>
</tr>
<tr>
<td>Action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td>31</td>
<td>88.6</td>
</tr>
<tr>
<td>Do Not Some</td>
<td>4</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Based on Table 2, these results indicate that there is an increase in knowledge both during the post-test by 4 respondents, namely 11.4%. Besides, these results indicate that there is an increase in good attitude during the post-test by 1 respondent, namely by 2.6%. Also, these results indicate that there is an increase in the act of not smoking during the post test by 2 respondents, namely 5.7%.

**Table 3: The Differences in Behavior of SMPN 1 Palu Students About Smoking Before and After GETAR Spiritual Intervention**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>0.000</td>
</tr>
<tr>
<td>Attitude</td>
<td>0.000</td>
</tr>
<tr>
<td>Action</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3 showed that because the p value = 0.000 (knowledge, attitude, and action). This means that there is a difference in the knowledge, attitude, and action of SMPN 1 Palu students about smoking before and after the GETAR spiritual intervention.
Table 4: The Difference in Behavior of Students Between Those Given Spiritual GETAR Intervention (Students of SMPN 1 Palu) and those Not Given Spiritual Intervention GETAR (Students of SMPN 4 Palu)

<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>0.000</td>
</tr>
<tr>
<td>Attitude</td>
<td>0.020</td>
</tr>
<tr>
<td>Action</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 4 showed that because the \( p = 0.000 \) (knowledge and action) and \( p = 0.020 \) (attitude). This means that there is a difference in the knowledge of students between those who were given GETAR intervention (students of SMPN 1 Palu) and those who were not given GETAR spiritual intervention (students of SMPN 4 Palu).

**Discussions**

Knowledge is the result of knowing, and this happens after someone senses a certain object\(^8\). Based on research results, GETAR spiritual intervention has been proven to increase students’ knowledge about smoking. GETAR intervention can be a differentiator in determining students’ knowledge about smoking.

In line with the research of Nuradita et al which shows that there is an effect of health education on knowledge about the dangers of smoking in adolescents at SMP Negeri 3 Kendal with \( p \) value = 0.000\(^9\). A research states that there are differences in the knowledge of adolescents before and after health promotion about the impact of smoking on school children at SMPN 1 Klari\(^{10}\). Fahrosi’s research shows that there are differences in the level of knowledge about the dangers of smoking in junior high school adolescents in rural and urban areas in Jember Regency. There are differences in the level of knowledge of adolescents in rural and urban junior high schools due to differences in information obtained from adolescents. The difference in media that can be accessed by teenagers is also a factor that affects the level of adolescent knowledge about the dangers of smoking. Other factors that cause differences in the level of knowledge include age and gender\(^{11}\).

Peer-led interventions are effective in increasing respondents’ knowledge regarding smoking. Continuing health education programs must be prepared in schools\(^{12}\). Sherman et al.bast research shows that pharmacy students and community leaders showed increased knowledge after smoking cessation educational interventions, and pharmacy students experienced an increase in self-confidence scores. Developing a coalition of health care providers and community leaders, with a focus on their respective roles, may be productive in initiating smoking cessation programs\(^{13}\). Ghrayeb research shows that health education is effective in increasing smoking knowledge. It is recommended that community-based health education programs and continuing schools about smoking should be implemented by health professionals\(^{14}\).

Attitude is a reaction or response that is still closed from a person to a stimulus or object\(^8\). Based on research results, GETAR spiritual intervention has been proven to increase students’ attitude about smoking. GETAR intervention can be a differentiator in determining students’ attitude about smoking.

In line with the research of Hidayati et al which showed that there was an influence before and after health education was carried out on attitudes about the dangers of smoking at YWKA high school in Palembang in 2019 (\( p = 0.000 \))\(^{15}\). A research shows that interventions in the form of counseling and giving leaflets to students has a good influence on students’ attitudes about smoking at SMK Negeri 2 Bitung City\(^{16}\). Sulastri & Rindu’s research states that there are differences in adolescent attitudes before and after health promotion regarding the impact of smoking on school children at SMPN 1 Klari\(^{10}\).

Chaaya et al. research’ suggests that educational campaigns, smoking cessation services and strict enforcement of policies may be needed to increase their effect further reducing student attitudes towards smoking\(^{17}\). A Research shows that peer-led interventions are effective at improving respondents’ attitudes regarding smoking. Continuing health education programs must be prepared in schools\(^{12}\). Girls are overall more positive for the attitude component of smoking prevention interventions. Our findings highlight the importance of considering differences in intervention
preferences for boys and girls in future health prevention initiatives\textsuperscript{18}.

Action or practice is an attitude that has not been automatically manifested in an action (overt behavior)\textsuperscript{8}. Based on research results, GETAR spiritual intervention has been proven to increase students’ action about do not smoking. GETAR intervention can be a differentiator in determining students’ action about do not smoking.

In line with the research by Puspitasari et al which shows that self-management training can reduce adolescent smoking behavior, namely 6.5 cigarettes in two weeks. The role of School Health Enterprises (UKS) must be increased by collaborating with educational institutions, especially school counseling teachers, in providing periodic smoking hazard education programs so that adolescents are able to reduce and or stop smoking and prevent other teenagers from having smoking behavior\textsuperscript{19}. To increase the motivation to quit smoking in adolescents, health education can be used in the form of media such as Facebook and leaflet media\textsuperscript{20}. Also, there are significant differences in the psychomotor actions of adolescents before and after being given peer education at SMAN “X” Denpasar. can be used in schools as a method of providing information through peer education to be more effective so that it can influence friends not to smoke and avoid smoking behavior\textsuperscript{21}.

Peer pressure-based interventions reduce the proportion of low-educated adolescents who start smoking. Therefore, influencing social norms and peer pressure would be a promising strategy for smoking prevention among adolescents. The results also suggest that additional interventions in subsequent years are needed to maintain their effect\textsuperscript{22}. Research by Bast et al shows that X: IT intervention has the potential to prevent cigarette absorption among adolescents. Intervention X: IT performed shows the overall effect of smoking prevention. Some of the interventions appear less attractive to children from low socioeconomic families\textsuperscript{23}. Research by Raji et al shows that peer-led interventions are effective at increasing respondents’ actions not to smoke. Continuing health education programs must be prepared in schools\textsuperscript{12}. Students show an overall positive attitude towards public action. Saudi Arabian teenagers have higher smoking rates than those in industrialized countries. They also have uneven knowledge about its negative effects, but show positive attitudes towards public actions on smoking\textsuperscript{24}.

**Conclusions**

Based on the results of this study, it concludes that there is a difference in the knowledge, attitudes, and actions of SMPN 1 Palu students about smoking before and after the spiritual intervention of GETAR. And there is a difference in the knowledge, attitudes, and actions of students between those who were given the spiritual intervention of GETAR (students of SMPN 1 Palu) and those who were not given the spiritual intervention of GETAR (students of SMPN 4 Palu).

**Conflict of Interest:** None

**Source of Funding:** Self

**Ethical Clearance:** Health Research Ethics Committee, Faculty of Public Health, Universitas Hasanuddin

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Teaching-Learning Styles & Aids Student’s Preferences among first Year Undergraduate Medical Students in the College of Medicine, University of Missan, Iraq

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Abstract

Objectives: to collect feedback from first year medical students on their perceptions and preferences of different teaching, learning, and aids methods that are currently used in medical chemistry teaching course. The aim of our study was to compare the more acceptable teaching & aids methods among the first stage medical students, Missan Medical College. So that best teaching aid and method can be adopted to improve their academic performance.

Materials and Methods: a well-structured electronic questionnaire based study was carried out on undergraduate medical students at department of clinical biochemistry, medical college, Missan University, Missan province, Iraq in second semester of academic year 2020-2021. 197 students participated out of 204 total, giving 97% response rate, the questionnaire was properly explained. The students were asked to grade teaching, learning, and aids methods that were used to teach Medical Chemistry module. The responses was analysed according to the Likert scale to detect their preferences and favourites toward the statements in the questionnaire.

Results: In our study many items with high satisfaction and least preferences grades was noticed for combination of teaching, learning, and aids methods. The majority (76%) of the students like the subject of medical chemistry. Exams preparation techniques, practical exercise lectures, various teaching aids and other methods have been found excellent and very good methods by the majority of the students. For effective teaching of medical chemistry, students opinions and preferences were suggesting a clear mandate in different issues such as usefulness of more preparations techniques for main exams, using more than one teaching aids (white board + power point presentation) in the lecture. By understanding students perceptions, teaching learning precesses in medical chemistry can be improved.

Keywords: Medical Chemistry, Biochemistry, teaching methods, Medical Student, learning style, Teaching Aids, First year.

Introduction

One of the essential components during the initial year in medical schools is the medial chemistry as a part of the Iraqi and worldwide curriculum. The medical chemistry course consisted of teaching analytical chemistry, organic chemistry, and basic biochemistry such as basic principles and applications of analytical chemistry in the medical field, and detection of inorganic elements by qualitative techniques, using specific reagents to distinguish between organic compounds evaluations acid-based concentrations by using titration methods as a quantitative technique. While In basic biochemistry students need to learn basic biochemistry objectives including carbohydrates, lipids, and proteins with its classifications and general properties. Normally the term of learning styles refers to the learning methods of gathering, organisation, and processing, interpreting think about information or to a set of factors including attitude, behaviour that makes the learning for individuals easier. In general identifying or knowing
the learning style is very important for understanding the differences amongst the learners and making the learning process stronger in the area of power and supporting the weaknesses in every style giving the learning tool better design and developments. According to Fleming (1995): Students who prefer visual learning are able to process the information best if they can see it with best understanding and learning from using materials presentation such as chart, graphs, pictures and diagrams on the other hand some students likes to get their learning through presenting and discussion of the information and they process it through attending tutorials, listening to the lectures and playback recorders of any learning sessions. Other types of students called “r” are learners who prefer to process information through reading and writing as well as some students also called kinaesthetic learners “k” better understanding of the information by experience and material practicing that have linked to the reality and they preferred the examples and the application to be from the concrete type every faculty members needs to address this concern by understanding the learning attitude and styles preference of their students where the learning style can be recognized as the manner and conditions under making the learner most efficiently and effectively perceives, stores, processes and recalls what they’re attempting to learn. Learning styles is a complex field with more than 70 different models and aspects and the most successful methods that meet the students preferences and likeness targeting better academic grades and performance. Furthermore, medical students currently represent a wide spectrum in regards of experience, ethnicity, age, culture and preparation level alongside with their learning preferences and styles. This diversity is more welcome as well as it challenges the instructor to meet the educational needs of all the students, where the student can improve with the learning preference and styles with the adopted instructions together. Therefore the instructor is responsible to be familiar with that diversity of learning styles and develop appropriate learning approaches.

Materials and Methods

This study was carried out among undergraduate first year medical students, the students were asked to complete the questionnaire and the students were informed that their participation in this study was voluntary. The questionnaire (25 statements) consisted of two parts, the first part was views or opinion questions about current teach-to-learn processes of current Medical Chemistry module and get the relevant information depending on the students’ responses to target future improvements and modifications. The second part of the questionnaire was about teaching learning methods & aids split into two halves (10 questions, five questions each) primary, regarding various learning styles like best methods exams or exam preparations and next concerning teaching aids and students preferences methods of delivering lecture such as, whiteboard, power point presenter, scientific videos, pictures and models. The Medical Chemistry modal was mainly twice a week (3h) and once a week for practical classes based on a pre-formatted time table. The aim of this study was explained to the students without unfolding the possible result as we asked the students in the beginning of this questionnaire about their names, sex. Then their responses were assessed by Likert type scale from 1 to 5 (Strong disagree, Disagree, Neutral, Agree, Strongly agree), only one question were on yes or no or neutral basis, this question is Do you like medical chemistry? The Microsoft excel worksheet was used to analyze the collected data.

Results

The questionnaire was consented and completed by total of 204 first stage medical students among them only 197 questionnaire responds were completely filled up and taken into analysis, consisting 74 males and 123 were female participate students. See figure 1.
In regards in the degree of liking and references to the medical chemistry module, the only question which was responded by all students was the first question and the results showed as following 76% of the students liked the subject of medical chemistry, 6% didn’t and 18% neutral.

Teaching learning methods & aids in Medical Chemistry

Various teaching learning methods were found by students preferences to be most popular, interactive, and very useful in the students minds such as monthly exams with a lesser topics, having an pre- exam (marks countless) before the main exams, preparing to the main exams through a lot of problems solving duties or homework’s, and using viva voce exams could facilitate understanding learning, as well as supersizing or prepared quizzes got a good percentages of Students’ acceptance and preferences as the Quizzes help students identify what they know and what they don’t know, and will give the students a better idea how they should grasping the materials and give them the motivation for more studying. See figure 2
The following part of the questionnaire outcomes was an overall assessment by encouraging the student’s to put their own observations related to the most appreciated and least appreciated method or aids, as useful suggestions was provided by the learners making the lecture session more interactive by inclusion of using educational materials in the medical chemistry lecture. The medical students of Missan medical college clearly preferred to use the educational method which is white board only\textsuperscript{17}. See figure 3.

![Graph showing opinion of 1st medical students towards the various teaching aids used for Conducting Medical Chemistry theory classes.](image)

On other hand, the whiteboard + PowerPoint presentation method was the most preferred method among first stage medical college and was much appreciated. Furthermore the questionnaire results present more affinity towards using diagram, picture, and videos in the lecture. From the main reason of preferring the method that contains whiteboard, this method can make the student & teacher interaction develop and there will be less chance of attention diversion, maintenance of a good eye contact and the contents are easy to understand, while with the PowerPoint method being in favour from students to this technique because it can provide text, diagram and images on the same slide with good quality, so it is clearly legible and more interesting\textsuperscript{18}. The medical students of Missan medical college clearly preferred to use the educational method the use of white board only, while the (whiteboard + PowerPoint presentation) method was the most preferred method among first stage medical college and was much appreciated. Furthermore the questionnaire results present more affinity towards using diagram, picture, and videos in the lecture. From the main reason of preferring the method that contains whiteboard, this method can make the student & teacher interaction become better and there will be less chance of attention diversion, maintenance of a good eye contact and the contents are easy to understand, while with the PowerPoint method being in favour from students to this technique because it can provide text, diagram and images on the same slide with good quality, so it is clearly legible and more interesting\textsuperscript{19}. 

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\textsuperscript{17} The study of medical chemistry for the first stage is considered useful and easy for first stage medical students.

\textsuperscript{18} Are first-stage students facing difficulty in the English language offered and translated in medical chemistry lectures?

\textsuperscript{19} Do your instructors give enough opportunity to clarify doubts of students in the Medical Chemistry lectures?

\textsuperscript{18} Do you think that the language of lectures presentation is simple to the student to learn?

\textsuperscript{19} Does the character and strength of the lecturers influence his improvement in his teaching method of medical chemistry?
Perceptions and opinions towards improving teaching learning of Medical Chemistry:

The results come out with High percentage of agreements and support from the students which were appeared from their responses related to how much beneficial for the first year students to study medical chemistry, the English language during learning process of medical chemistry is difficult, chances obtaining and enough time was given to the students to get the answers for their questions, the scientific language that is used in the medical chemistry lectures simple to learn and understandable, there much effect of lecturer characters strength on the students which improve his teaching methods. See figure 4.

![Figure 4: Perceptions and opinions towards improving teaching learning of Medical Chemistry](image)

Additionally, the collected data was also shown the student approval and acceptances to supporting summaries of the topics should be given at the beginning of medical chemistry lectures, outlined concepts are understandable to the learner as the summarization of key concepts at the end of the lectures is so important, while the responses in regard the division of Medical Chemistry subject into three sections which consist of Biochemistry, Analytical, and organic chemistry was displayed the most students felt that that division is necessary, for better understanding of the Medical Chemistry various and interesting aspects, also, students feel that the practical side of medical chemistry is very valuable where the experiments are an essential part of the undergraduate medical curriculum, See Figure 5.
Discussion

Student’s perceptions and views in the present study about their experience toward the teaching learning methods & aids during the first year of study in Missan (Government) university, medical college, were documented. The publications on the student’s preferences and opinions about medical chemistry objective very less. Currently the medical chemistry module is being taught of 1 year in medical colleges of Iraq. However the issues that are discussed in this article and the students perceptions about it are unlikely to differ significantly with the other countries even in case of different curriculum or in case of a longer duration of the cause.

Conclusion

The feedback that are given by the students was very helpful and had facilitate a change about the currently used teaching learning and aids principles in the preconceived notions and their opinions and preferences needs to be taken into consideration to fill the gaps between and the current medical education system and to make the teaching learning processes more meaningful and students friendly. Recommendation, will be very useful to the teaching learning & aids community to do further studies on validating the students perception & preferences. The results of our survey reports, it’s showed there was difference of perception towards teaching methods between the students. This study actually highlighted some students preferred learning methods &
aids clearly confirmed that a good understanding of the lecture doesn’t rely on teaching methods but it depends on the teacher. Because if the teacher is attentive and deferential and well trained any method he uses would be effective, teaching is the quality of a teacher not the quality of teaching technology. In teaching technologies formal training is needed to improve the presentation leads the student for more interactive, motivation and high level of academic performance.  

Acknowledgements: Thanks and appreciation to all undergraduate first stages medical students, Medical college, Missan University, academic year 2020-2021, for their perfect collaboration and participation to answer the questionnaire statements and expressing their suggestions with preferences to have more interactive and informative lectures.

Ethical Clearance- Taken from Medical college committee / University of Misan/mcm@uomisan.edu.iq

Source of Funding- Dr Mustafa Alyassiry ONLY

Conflict of Interest - Nil

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The Exposure of Gap Competence causes in Stunting Children Ages 0-12 Months in Makassar Indonesia: Cross-Sectional Study

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Abstract

Introduction, The parenting gap is believed to be one of the variables that influence stunting. The Objectives of this study to identify gaps in care (feeding practices, care, hygiene, and treatment of children) and incidence of stunting. Method this study is a cross-sectional, conducted in March-June 2020 in Makassar City Indonesia. The sample size was 82 people, of children aged 0-12 months. The data collectors are alumni of applied nutrition and dietetics degrees, trained for 2 days in health protocol, interviews, and anthropometry. The instrument in this study was developed from the UNICEF. The result, found that mothers and fathers graduated from high school, 43% and 59.8%, respectively. The work of mothers as housewives is 84.1% and fathers are private employees as much as 36.6%. The parenting competency gap in the feeding practice of children aged 0-6 months, 7-9 months, and 10-12 months is 20.9%, 71.79%, and 92.31%, respectively. The results of statistical analysis showed that there was an effect of parenting competency gaps on stunting ($p = 0.000$). The conclusion is that the parenting competency gap is proven to affect the incidence of stunting. The suggestion is that it is necessary to increase the capacity of mothers in child care practices.

Keywords: Parenting Competency Gap, Stunting

Introduction

Currently, the most serious nutritional problem in Indonesia is stunting. The national prevalence of stunting is 36% and South Sulawesi reaches 30.8%. The impact caused by stunting is low academic potential, high risk of non-communicable diseases, high-cost burdens on health services, and low productivity. The problem of stunting must be prevented because the incidence of stunting from birth is difficult to treat. This means that starting from pre-pregnant and pregnant should intervene with nutrient-rich foods of local potential (1), (2), (3).

Prevention of stunting is best done through the consumption of foods of sufficient quality and quantity during pregnancy up to 2 years (3), (4). The problems faced in meeting the quality and quantity of food according to nutritional needs are income, preferences, availability, and nutritional knowledge. Sufficient income guarantees food diversification and food availability. The problem is only in consumer preferences, which limit a variety of food choices, including children’s food. If the caregiver does not understand how to feed the child, the effect was low consumption of nutrients. The longer this happens, the exposure, the greater the risk of stunting (5), (6), (4).

Maternal competence is not only feeding practice but also hygienic practice and child care practice (7), (8), (9). This competency component should be owned by mothers before having children. In this context, it stands to reason that maternal nutritional literacy is urgently needed (10). Studies are needed to measure the effect of...
the competency gap above the risk of stunting.

**Objectives**

Analyzing the effect of competency gaps on stunting of children aged 0-12 months

**Methods**

**Design and Procedure**

The research design was a survey with a cross-sectional study. The sample size is calculated based on the following sampling formula (11). Based on the sampling formulation above, it was determined that the sample size of the mother was 84 people. Procedure data collected; (1) 2-day Enumerator Training with materials on Health Protocols for the Prevention of COVID-19, Research Interview and Anthropometric Measurement, (2) Field Officer Coordination Meeting with Enumerators, (3) Selection and randomization of participant numbers between 0-12 mo. All enumerators are provided with a mask and a hand sanitizer as well as for all nannies. Data collection was carried out for 7 days with details of 10 enumerators with a target number of 82 people or the equivalent of 10 people and one enumerator for the seven days of the survey. Supervisors make field visits to ensure that health protocols are carried out properly.

**Statistical Analysis**

To Analysis of the effect of GAP on Competence with Stunting tested by the Chi Square test. The competency gap in child feeding practice is measured by the frequency, amount, consistency, and variation of child feeding compared to the fact of child feeding with the standard of child feeding by UNICEF with indicators. And The ability of caregivers to know the concept of child care and implement it in child care according to the principles of child care includes the use of cleaning tools (soap), washing hands, using child masks, mother masks, masks of other family members, bathing and washing hair. The score for caring practices was 6-14 pints of the 7 question items.

Competency of hygiene practice can be defined as the ability of caregivers to know the concept of children’s hygiene and sanitation and implement it in children according to the principles of individual hygiene includes bathing children, stimulating toilet training, and using foot mats. The hygiene practice competency score is 5-9 points from 4 question items.

Health seeking behavior competence can be defined, the ability of caregivers to know the right treatment-seeking concept implements it in the pattern of seeking treatment for children when they are sick. The medical competency score is 1-2 of 1 question.

**Results**

**Characteristics of Respondents**

**Table 1. Characteristics Subjects**

<table>
<thead>
<tr>
<th>Demography</th>
<th>Father</th>
<th></th>
<th>mother</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ elementary school</td>
<td>8</td>
<td>6.1</td>
<td>5</td>
<td>6.1</td>
</tr>
<tr>
<td>§ junior high school</td>
<td>10</td>
<td>12.2</td>
<td>10</td>
<td>12.2</td>
</tr>
<tr>
<td>§ Tama High School</td>
<td>49</td>
<td>59.8</td>
<td>31</td>
<td>37.8</td>
</tr>
<tr>
<td>§ College</td>
<td>18</td>
<td>22.0</td>
<td>36</td>
<td>43.9</td>
</tr>
<tr>
<td>§ Sub-total</td>
<td>82</td>
<td>100</td>
<td>82</td>
<td>100</td>
</tr>
</tbody>
</table>
Occupation

| §   | Workers | 26 | 31.7 | 69 | 84.1 |
|§   | government employees | 19 | 23.2 | 5 | 6.1 |
§   | Traders | 7 | 8.5 | 2 | 2.4 |
§   | General employees | 30 | 36.6 | 6 | 7.3 |
§   | Sub-total | 82 | 100 | 82 | 100 |

Based on the results of this study it is known that in general, the mother’s education is higher than that of the father. Father and mother’s college education is 22% and 43.9%. Based on the results of this study, it is known that the father’s job is as a private employee 36.6%, and the mother as a housewife 84.1%.

Parenting Competencies

<table>
<thead>
<tr>
<th>Patenting Competencies</th>
<th>Categories</th>
<th>0-6 mo n (%)</th>
<th>7-9 mo n (%)</th>
<th>10-12 mo n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding Practice</td>
<td>Lack of</td>
<td>9</td>
<td>20.93</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>34</td>
<td>79.07</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>43</td>
<td>100</td>
<td>39</td>
</tr>
<tr>
<td>Caring Practices</td>
<td>Lack of</td>
<td>29</td>
<td>67.4</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>14</td>
<td>32.6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>43</td>
<td>100</td>
<td>27</td>
</tr>
<tr>
<td>Hygiene</td>
<td>Lack of</td>
<td>25</td>
<td>58.1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>18</td>
<td>41.9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>43</td>
<td>100</td>
<td>27</td>
</tr>
<tr>
<td>Health Seeking Behavior</td>
<td>Lack of</td>
<td>05</td>
<td>11.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>38</td>
<td>88.4</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>43</td>
<td>100</td>
<td>27</td>
</tr>
</tbody>
</table>

Based on the results of the study, it is known that at the age of fewer than six months or during the exclusive breastfeeding period, the competence of mothers in giving food is still good because only by paying attention to breastfeeding properly.
Nutritional status

Table 3. Child Nutritional Status

<table>
<thead>
<tr>
<th>Nutritional Status</th>
<th>Categories</th>
<th>0-6 mo n (%)</th>
<th>7-9 mo n (%)</th>
<th>10-12 mo n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunting</td>
<td>Severely Stunted</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Stunted</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>36</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>43</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>48</td>
<td>34</td>
<td>82</td>
</tr>
</tbody>
</table>

Based on the results of this study, it is known that the percentage of children who are stunted (very short and short) increases with age. Stunting in the 0-6 months, 7-9 months, and 10-12 months age groups were 16.3%, 14.8%, and 25.7%, respectively.

Parenting Competency Status Gap

Table 4. Parenting Gap Competencies

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not real Gap Competencies (NRGC)</td>
<td>48</td>
<td>58.5</td>
</tr>
<tr>
<td>Real Gap Competencies (RGC)</td>
<td>34</td>
<td>41.5</td>
</tr>
</tbody>
</table>

Based on the study results it is known that the gap in childcare competence is not really as much as 58.5% and very real as much as 41.5%.

Effect of Competency Gap on Stunting

Table 5. Parenting Gap with Child Stunting

<table>
<thead>
<tr>
<th>Status</th>
<th>The Status of Gap Competencies</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRGC n (%)</td>
<td>RGC n (%)</td>
</tr>
<tr>
<td>Stunting</td>
<td>2 (2.4)</td>
<td>12 (14.6)</td>
</tr>
<tr>
<td>Normal</td>
<td>46 (56.1)</td>
<td>22 (26.8)</td>
</tr>
<tr>
<td>total</td>
<td>48 (58.5)</td>
<td>34 (41.5)</td>
</tr>
</tbody>
</table>

Based on the results of this study, it is known that there is an effect of competency gaps on the incidence of stunting in children aged 0-12 months (p = 0.000)
Discussion

The important outcome in this study is the gap in the competence of care for feeding, hygiene, care, and treatment. The gap in the competence of child feeding practices for caregivers found in this study is that the older the age, the feeding gap becomes more pronounced because the percentage of children who are not good at feeding practices increases from 20% to 92.3% at age 0-6 months to 10-12 months of age. Almost the same as the gap in children’s caring practices competence for caregiver mothers, the percentage of mothers with poor care practices from 67.4% at 0-6 months of age, and an increase of 10-12 months to 75%. The gap in children’s hygiene competence for caregivers is the only competency that is getting better day by day from 41.9% good status at 0-6 months to 50% in the 10-12 month age group. The gap in children’s health-seeking behavior for caregivers, in general, is very good 100% at 10-12 months of age and only 88.4% is good in the 0-6 month age group. The results of the analysis of the effect of the influence of the competency gap on stunting of children aged 0-12 months were found that there was an effect of the parenting competency gap on the incidence of stunting in children aged 0-12 months.

The results of previous studies that are in line with the findings in this study are research conducted by Sirajuddin, Nursalim, et al (2020), which states that the practice of complementary feeding that is owned by mothers is very important to prevent stunting. The research of Sirajuddin et al. (2000) explained that the way mothers feed their children, especially complementary foods, affects the incidence of stunting. This study also found evidence that overall parenting competence is also an integral part of child feeding practices. Competence in feeding practice is in line with a study by Sukmawati and Sirajuddin,(2020) when children eat, ideally, they should still be accompanied by the mother, not only those who are still being fed, but also for children who can feed themselves without being fed.

The competence of child feeding care is related to the mother’s ability to continue to breastfeed exclusively and also to overcome all breastfeeding problems. This condition is very beneficial at the same time there is a threat of decreasing purchasing power due to the COVID-19 pandemic. For mothers who have decided to wean their children, of course, they will face purchasing power (14). It is suspected that some families affected by the pandemic have lost their jobs and daily income (Pérez-Escamilla, Cunningham, and Moran 2020). However, there are advantages for those who are still exclusively breastfeeding, as evidence from previous studies that in poor families, breast milk is very helpful in supplying children’s needs so that they are not prone to becoming stunted, especially in the first 1000 days of life (12).

A longitudinal study conducted in Cambodia in 2020, found that competence in child feeding parenting should increase in each age group. Mothers who can increase their capacity in childcare based on the child’s age can assure that their child’s nutritional consumption will be fulfilled. The practice of feeding children based on 4 indicators of child feeding globally was not taken seriously in this study by caregivers (15). The same study also reported from Guatemala, 2014 that the indicators of the quality of child feeding always differ between regions. It is desirable that observations that are not only carried out quantitatively on these 4 indicators but should also be combined with a qualitative approach. This is aimed at exploring local practices as indigenous customs of the surrounding communities. The use of local food ingredients and special methods that have developed naturally in the community (16). Improvements in nutritional status, especially stunting, not only pay attention to nutrient intake but also hygiene and sanitation as factors that directly affect children’s stunting status. (16), (17),(18).

The limitations of this study are that it cannot make intensive observations on the practice of parenting. This is due to the limited access to house visits by enumerators due to the social distancing policy that was enforced during the COVID-19 pandemic in Makassar City in April-June 2020. Enumerators have been trained to interview before the interview was conducted. Several attempts were made by researchers to anticipate the weaknesses of this study.

The generalization (generalizes) in this study is that there is very strong evidence of gaps in parenting competence to mothers. This gap increases with increasing children’s age. The reason is that there is no effort to increase the capacity of mothers on how to care...
for children based on age groups. Ideally, the capacity of mothers will be improved with increasing age of the child, because the complexity of care is greater and requires seriousness in every childcare practice.

**Conclusions**

The effect of the competency gap on the stunting of children aged 0-12 months is that the more real the competency gap is, the greater the risk of children becoming stunted. Capacity building for caregivers is needed according to the child’s age. Capacity building in the practice of feeding and caring for children through mentoring activities at the household level.

**Ethical Clearance:** Taken from the Ethics Commission of Makassar Health Polytechnic Indonesia

**Sources Founding:** Sources Founding from Health Polytechnic of Makassar, Indonesia.

**Conflict of Interest:** Author declare no conflict of interest in this study.

**References**


The Interleukin-6 (rs1800797) Variant in Healthy Individuals at Medan City, North Sumatera Province

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Abstract

The Interleukin-6/IL6 (rs1800797) variant play a role in several diseases pathogenesis. The frequency genotype of IL6 (rs1800797) varied based on different race, ethnicity, and underlying character in population. This study aimed to assess the frequency of genotype and allele of IL6 (rs1800797) in healthy individuals at Medan city, North Sumatera Province Province and this study can be used as a reference in determining of several diseases for further studies. Genotyping of IL6 (rs1800797) in the promoter region using polymerase chain reaction and restriction fragment length polymorphism method, analysis using FokI restriction enzyme. This study showed that the genotype frequency of GG, GA, and AA were 95.8 %, 4.2 %, and 0 %, respectively. The allele frequency of G and A were 97.9 % and 2.1 %, respectively. The results indicated that the homozygous wild-type (GG) of IL6 (rs1800797) was higher than the heterozygous mutant (GA) and there was absent of homozygous mutant (AA) in this population. The frequency of G allele also higher than A mutant allele.

Keywords: Interleukin 6 (rs1800797), variant, gene, polymorphism, healthy

Introduction

The Interleukin-6/IL6 (rs1800797) gene variant play a role in several diseases pathogenesis such as febrile seizures, type 2 diabetes mellitus (T2DM), diabetic nephropathy, hyperandrogenism, cancer, and etc.1-5 IL6 is a pleiotropic cytokine that consists of 212 amino acid with molecular weight ranging from 21 to 28 kDa. The gene of IL6 gene is located on chromosome 7p21, consisting of four introns and five exons, and there are some of very rare polymorphisms in this sequence. The IL6 (rs1800797) gene polymorphism consist of single nucleotide change from G (guanine) to A (adenine) at position -597 in the promoter region. IL6 (rs1800797) gene was found three variant such as homozygous wild-type (GG), heterozygous mutant (GA) and homozygous mutant (AA).6,7

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The frequency of the rs1800797 mutant variant varied based on different race and ethnicity in population and character that underlies that population. The previous study of rs1800797 showed that the frequency of homozygous mutant (AA) was not found in the healthy subject of Chinese and Thailand population,8,9 differ from the genotype frequency of Czech and Swedish population.10,11 Association of genotype or allele of IL6 (rs1800797) with several diseases also known to vary according to race or ethnicity. In a meta-analysis by Wang et al. (2019) showed IL6 (rs1800797) gene variant may be associated with an increased risk of liver diseases in the non-Asian population.12 Other meta-analysis showed that cancer diseases such as breast cancer, non-Hodgkin’s lymphoma, B-cell lymphoma and diffuse large B-cell lymphoma in Caucasian but not in Asia population were associated with IL6 (rs1800797) gene variant.5

Base on genotypes of the IL6 (rs1800797) gene variant may vary according to race or ethnicity on the differences in population and also on the underlying
characters, and the variant genotype may be involved in several diseases, so the present research aimed to find out assess the frequency of genotype and allele of IL6 (rs1800797) in healthy individuals among Medan city, North Sumatera Province. We hope, these results study can be used as a reference in determining of association of IL6 (rs1800797) with several diseases for other next studies by other researchers.

**Materials and Methods**

**Ethics**

This study was carried under the ethical provision of the Declaration of Helsinki. The study was approved by the Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara (No. 447/KEPK-FKUSU-RSUPHAM/2019 Informed consent was taken from the participant that agree to participate in this study.

**Study Design**

This study was an observational with a cross-sectional study design. Healthy subjects in this study were gym participants from several gyms in Medan city, North Sumatera Province, staffs and students in the Faculty of Medicine, USU. Inclusion criteria were male or female, aged 20-65 years, and fasting blood glucose <126 mg/dl. However, subjects who have malignant disease were excluded from this study.

**Genotyping of The IL6 (rs1800797)**

Analysis of IL6 (rs1800797) variant was carried out in Molecular Biology Integrated Laboratory at Medical Faculty of Universitas Sumatera Utara (USU) from March to December 2019. DNA isolated from leukocyte using isolation kit and DNA purification (Promega, USA).

Polymerase chain reaction (PCR) process in IL6 gene amplification using primer (F) 5’- GGA GTC ACA CAC TCC ACC T -3’ (R) 5’-CTGATT GGAAACCTTATTAAG-3.13 PCR reaction solution consists of primer forward one μL, primer reverse one μL, PCR master mix 12.5 μL, dH2O steril 8.5 μL, DNA template two μL. PCR program carried out with pre-denaturation at 95°C for 4 minutes, then denaturation at 95°C for 30 seconds (30 cycles), annealing at 57°C for 30 seconds, extension at 72°C for 30 seconds, continued by a final extension at 72°C for 5 minutes.13 PCR product found at 525 bp and continued the digestion by FokI enzyme (ThermoFisher). The RFLP visualization result using UV transilluminator (Gel Doc, BIO-RAD Laboratories USA), were GG genotype (525 bp), GA genotype (525 bp, 468 bp, 57 bp), AA genotype (468 bp, 57 bp).

**Statistical Analyses**

Frequency of IL6 (rs1800797) variant was displayed descriptively after calculated by direct counting. Distribution of genotypes and alleles were analysis using SPSS program version 22.

**Results and Discussion**

PCR-RFLP products of IL6 (rs1800797) showed in Figure 1 and 2.

IL6 (rs1800797) variant distribution of one hundred and twenty healthy subjects in this study showed in Table 2.
The present study showed that the homozygous wild-type (GG) of IL6 (rs1800797) was higher than heterozygous mutant (GA) (95.8% vs 4.2%) and there was absent of homozygous mutant (AA) in this population. The frequency of G allele also higher than A mutant allele (97.9% vs 2.1%).

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that has been implicated in various conditions such as cardiovascular disease, diabetes mellitus, etc. Circulating levels of IL-6 may be influenced by common genetic variants of the IL-6 gene promoter. Several IL-6 gene SNPs in the IL-6 promoter region have been identified, such as SNP rs1800797 (-597 G/A or -598 G/A). The IL6 (rs1800797) was found three variants. The genotypes and mutant alleles could be vary based on race and ethnicity of the population of study and the underlying character. Ethnic differences were found in IL-6 (rs1800797) variant.

The present study has conducted to analyze the variant distribution of IL6 (rs1800797) in the healthy population at Medan city, North Sumatera Province. Most population in this city is dominated by Batak and Melayu ethnic. This study showed GG genotype of IL6 (rs1800797) was higher than GA genotype (95.8% vs 4.2%). The previous studies in healthy population of Chinese, Tunisia, Brazil, and Egypt showed the same results, i.e. GG genotype higher than GA genotype. This present study showed absent of AA genotype. Other previous studies were found the same where the AA homozygote of IL6 (rs1800797) promoter region completely absent in healthy Thailand and Chinese population.  

Our study also showed that G allele was higher than A allele, this was in line with the previous study in healthy population of Thailand, Chinese, Tunisia, Brazil, and Egypt. But, in India, there was found A allele higher than G allele. Studies in several countries in the world showed the difference in distribution frequency of genotypes (GG, GA, AA) and alleles (G and A) of the IL6 (rs1800797) in the healthy population (Table 3).

### Table 2. Genotypes and alleles distribution of the rs1800797

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>115</td>
<td>95.8</td>
</tr>
<tr>
<td>GA</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>235</td>
<td>97.9</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>2.1</td>
</tr>
</tbody>
</table>

### Table 3. Genotypes and alleles frequency of IL6 (rs1800797) in healthy control among different populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Number investigated (N)</th>
<th>Genotypes frequency (%)</th>
<th>Alleles frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>In this study</td>
<td>120</td>
<td>95.80</td>
<td>4.20</td>
</tr>
<tr>
<td>Chinese [17]</td>
<td>232</td>
<td>100.00</td>
<td>0</td>
</tr>
</tbody>
</table>
To date, experts suggest that several diseases were associated with IL6 gene polymorphism. IL6 (rs1800797) increased the risk of atherogenesis and thrombosis in several diseases, such as coronary artery disease, deep vein thrombosis, acute ischemic stroke. IL6 (rs1800797) also play a role in the pathogenesis of several types of cancer, there were found a significant association between IL6 (rs1800797) variant with breast cancer, non-hodgkin’s lymphoma, but not gastric cancer.

Association of distribution of IL6 (rs1800797) variant with several diseases are known to vary according to race or ethnicity. In the meta-analysis by Wang et al. (2019) showed IL6 polymorphism (rs1800797) may be associated with an increased risk of liver diseases in the non-Asian population. Other meta-analysis showed IL6 (rs1800797) variant associated with several cancer such as breast cancer, non-Hodgkin’s lymphoma, B-cell lymphoma and diffuse large B-cell lymphoma in Caucasian but not in Asia. Other study showed that IL6 (rs1800797) associated with T2DM in the German population but did not associated in North India population. A study by Phillips et al. (2010) showed that IL6 (rs1800797) associated with metabolic syndrome in French populations, but Boeta-Lopez et al. (2017) showed that there was no association between IL6 (rs1800797) with metabolic syndrome in Mexican-Americans of South Texas population.

Further studies are needed in a larger population to analyze IL6 variation based on ethnic and its association with susceptibility of diseases.

### Conclusion

The present study concluded that GG genotype of IL6 polymorphism was higher than GA, and there is absent of AA genotype in this population. The frequency of G allele also higher than A mutant allele. This study has differences and similarities with previous studies in other countries, and it was possible because the ethnicity in each country varied. This results study can be used as a reference in determining of association of IL6 (rs1800797) with several diseases for another next study by other researchers.

### Acknowledgements and Funding:

This study was supported and funded by the Ministry of Research and Technology and the Higher Education Republic of Indonesia, Penelitian Dasar Unggulan Perguruan Tinggi of the year 2019 (Grant No: 21/UN5.2.3.1/PPM/KP-DRPM/2019).

### Transparency Declaration

**Conflicts of Interest:** Nothing to declare

### References


Effect of \textit{Malus sylvestris} Extract on Histopathological Features of Hypercholesterolemic Wistar Rat (\textit{Rattus norvegicus}) Fatty Liver

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Abstract

Objective: To evaluate the effect of \textit{Malus sylvestris} extract on improving the degree of steatosis and portal inflammation histopathological features of hypercholesterolemic \textit{Rattus norvegicus} strain wistar. Method: Forty-two male rats were divided into 6 groups randomly. Hypercholesterolemic fatty liver induced by giving high-fat diet (HFD) for 46 days on groups 1 to 5, while group 6 given standard diet with the same amount of time. Simvastatin was administered in group 2 at a dose of 0.36 mg/day. Groups 3, 4, and 5 were given \textit{Malus sylvestris} extract (MSE) as a treatment at doses of 90 mg/day, 180 mg/day, and 360 mg/day in sequential order. Simvastatin and MSE were administered for 14 days, from day 33 to 46. On day 47, all rats were sacrificed and the liver was removed for histopathological slides preparation with hematoxylin-eosin (HE) staining. Histopathological results were analyzed using Kruskal Wallis test followed by Mann Whitney test. Result: Histopathological analysis showed that \textit{Malus sylvestris} extract improved steatosis and portal inflammation features compared to HFD-fed rats in group 1 (p<0.05). Conclusion: \textit{Malus sylvestris} extract improved the degree of steatosis and portal inflammation histopathological features of hypercholesterolemic rat fatty liver.

Keywords: Fatty Liver, Histopathological, Hypercholesterolemic, \textit{Malus sylvestris}, Rat.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the accumulation of excess fat in the liver with more than 5\% of hepatocytes containing visible lipid vacuoles or steatosis affecting at least 5\% of the liver weight without heavy alcohol consumption or other secondary causes\textsuperscript{1}. The disease can progress from harmless simple non-alcoholic steatosis (NAS) to non-alcoholic steatohepatitis (NASH), a form of inflammation that damages the liver cells\textsuperscript{2}. Liver cell damage then can lead to fibrosis, cirrhosis, and eventually hepatocellular carcinoma (HCC), which require liver transplantation\textsuperscript{3}. Although cirrhosis due to hepatitis C is the leading cause of liver transplantation in the United States, NAFLD rank the second. As prevalence continues to increase, NAFLD will become major health problems and the major cause of liver transplantation in the future\textsuperscript{4}. This will also increase the demand for liver transplantation with fewer good-quality organs, as more donors have steatotic livers\textsuperscript{5}.

NAFLD has emerged as the most common chronic liver disease in developed countries. However, the prevalence of NAFLD continues to increase even in developing countries due to worldwide epidemic of obesity and other metabolic syndromes\textsuperscript{6}. The prevalence of NAFLD worldwide is approximately 25.24\%, with highest prevalence in the Middle East (31.79\%) and South America (30.45\%)\textsuperscript{7}. In Asia, there is almost 10\% increase in prevalence from the initial 25.28\% in 1999-2005 to 33.90\% in 2012-2017\textsuperscript{8}. The data have shown that the cases will always increase and become a problem not only in Western countries but also in Asia.
due to urbanization. Urbanization will lead to sedentary lifestyle with excessive dietary consumption resulting in obesity, a risk factor for NAFLD. Apart from obesity, NAFLD also accompanied by hyperlipidemia, hypertension, type 2 diabetes, cardiovascular disease, and other metabolic syndromes that may affect not only the liver but also the person’s systemic condition.

Environmental factors play an important role in the development of NAFLD, such as eating habits, daily activities, and socioeconomic factors. Dietary patterns of high sodium and fat with low consumption of fresh fruit have been found in NAFLD patients. By eating fruits and vegetables, especially apples, can reduce the risk of NAFLD due to fiber and chemical contents in apples such as flavonoids, polyphenols, and carotenoids, which have antioxidant and anti-inflammatory effects to prevent and protect the liver from NAFLD. So far, there are only few studies regarding to the effect of apples on treating NAFLD and there are no studies specifically using Malus sylvestris. Hence, this study aimed to evaluate the effect of Malus sylvestris extract on improving histopathological features of hypercholesterolemic Wistar rat (Rattus norvegicus) fatty liver.

Materials and Methods

Malus sylvestris collection and extraction:

Malus sylvestris were collected from apple farm in Junggo Village, Bumiaji, Batu, Indonesia. The apples were washed, drained, and weighed. Malus sylvestris with their skins were cut into thin strips and air-dried in the shade. To maximize the drying process, apple slices were roasted at 45°C for about 48 hours or until completely dried. The dried apple slices were crushed in a blender and sieved into powder. Malus sylvestris powder then extracted with 96% ethanol using percolation technique. Extraction process was continued with rotary evaporator to removed the alcohol content and a thick extract will be produced.

Experimental Animal:

Forty-two male Wistar rats around 2-3 months old weighing between 125-200g were used for the study. They were cared for in clean well-ventilated cages with light/dark cycle 12 h respectively and were given water and either a standard or high-fat diet (HFD) ad libitum. Before the experiment began, they will be acclimatized for seven days with a standard diet in the Experimental Animal Unit of Pharmacology Laboratory within Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Experimental design:

Forty-two Wistar rats were randomized and divided into 6 groups each containing 7 rats. Group I received HFD served as negative control rats. Group II received HFD and simvastatin at dose of 0.36 mg/day served as positive control rats. Group III received HFD and Malus sylvestris extract at dose of 90 mg/day. Group IV received HFD and Malus sylvestris extract at dose of 180 mg/day. Group V received HFD and Malus sylvestris extract at dose of 360 mg/day. Group VI received standard diet served as normal control rats. Standard or high-fat diet for the rats was given for 46 days. Simvastatin and Malus sylvestris extract (MSE) were administered orally by using oral gavage for 14 days (day 33-46). On day 47, all animals were euthanized using chloroform and sacrificed by cervical dislocation. The liver was removed for histopathological analysis.

Histopathological studies:

All livers were fixed by immersing it in 10% neutral buffer formalin for 24 hours. Histopathological preparations continued with dehydration, clearing, impregnation, and then making paraffin blocks, which were cut by microtome with thickness of 4-5 μm. The tissue slices were attached to glass object and then stained with hematoxylin-eosin (HE). Histopathological slides were observed per field of view under light microscope at 100x magnification to determine the observed area and 400x magnification to observe the cells more clearly. Observation of liver cells used semi quantitative scoring system to evaluate steatosis and portal inflammation features.

Statistical Analysis:

Statistical analysis was performed using SPSS 20 for windows and the results were represented as mean. All results were analyzed statistically by Kruskal Wallis test, followed by Mann Whitney test to determine the significant difference between groups.
Result

The results were considered to be statistically significant when p<0.05. Mean rank value of steatosis and portal inflammation from statistical analysis can be seen in Table 1.

### Table 1: Effect of MSE on histopathological features of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Histopathology Features</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.71a</td>
<td>19.86b</td>
<td>22.64b</td>
<td>18.00b</td>
<td>17.07b</td>
<td>14.29b</td>
</tr>
<tr>
<td>Steatosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal Inflammation</td>
<td>34.86a</td>
<td>20.14b</td>
<td>20.14b</td>
<td>18.33b</td>
<td>14.71b</td>
<td>17.43b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are represented in mean. Different superscript letters indicate significant difference (p<0.05).

Under the microscope, rats in normal control groups showed normal liver histology with minimal steatosis and portal inflammation features (Fig. 1F and 2F). Rats fed with HFD in positive control group showed severe fat vacuoles accumulation (steatosis) in liver cell cytoplasm (Fig. 1A) as well as inflammatory cell infiltration in portal area (Fig. 2A). However, Fig. 1B and 2B showed reduced fatty change and inflammatory cell in the portal area in simvastatin group, this suggest that the drug could alleviate steatosis and portal inflammation. All doses of MSE also showed similar result as simvastatin group by improving steatosis (Fig. 1C, 1D, 1E) and portal inflammation (Fig. 2C, 2D, 2E) features compared to positive control group.

![Figure 1](image-url)

**Figure 1. Effect of MSE on improving steatosis features in liver histopathology**

(A) Liver tissue of HFD induced rats. Black arrows indicate fatty vacuoles in liver cell cytoplasm (steatosis). (B) Liver tissue of rats treated with simvastatin. (C) Liver tissue of rats treated with MSE at doses of 90 mg/day. (D) Liver tissue of rats treated with MSE at doses of 180 mg/day. (E) Liver tissue of rats treated with MSE at doses of 360 mg/day. (F) Liver tissue of normal control rats.
Figure 2. Effect of MSE on improving portal inflammation features in liver histopathology

(A) Liver tissue of HFD induced rats. Black arrows indicate inflammatory cells that fill the portal area. (B) Liver tissue of rats treated with simvastatin. (C) Liver tissue of rats treated with MSE at doses of 90 mg/day. (D) Liver tissue of rats treated with MSE at doses of 180 mg/day. (E) Liver tissue of rats treated with MSE at doses of 360 mg/day. (F) Liver tissue of normal control rats.

Discussion

Accumulation of lipid droplets in liver cells can be macrovesicular or microvesicular, which is rich in triacylglycerol (TAG). Liver does not store TAG in normal conditions, but exposure to stress due to excessive intake of fat or carbohydrates such as HFD consumption may cause fat accumulation in the liver. This is associated with lipotoxicity because it increases mitochondrial stress. Mitochondrial dysfunction increased reactive oxygen species (ROS) production, which reduces antioxidants that act as a defense against oxidative stress in the liver. Ongoing oxidative stress may lead to lipid peroxidation that causes lesions to liver cells leading to degeneration and necrosis. The end products of lipid peroxidation, malondialdehyde, have chemoattractant properties that activate stellate cells as collagen producers and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) that activates c-Jun N-terminal kinases (JNK) pathway and nuclear factor kappa light chain enhancer of activated B cells (NF-κB), induce the release for more pro-inflammatory cytokines mediating liver inflammation. This may lead to NASH with mixed lesion of necrosis, inflammatory infiltrates, and fibrosis, in addition to steatosis.

This study showed that MSE give hepatoprotective effect by improving steatosis and portal inflammation features compared to rats fed with HFD. This might be due to the presence of polyphenols and pectin in Malus sylvestris, which have metabolic regulatory, antioxidant, and anti-inflammatory properties. Polyphenols reduce TAG accumulation by various mechanisms, including inhibition of lipogenesis and promotion of fatty acid catabolism by down-regulating sterol regulatory element-binding protein 1c (SREBP-1c), which has a major role in lipogenesis. Dietary fiber has also been shown to reduce the risk of NAFLD. Pectin is one of the dissolved dietary fibers found in apple in high concentrations, which increase the amount of intestinal microbiota that ferments pectin into short chain fatty acids (SCFAs) in large intestine. SCFAs containing 2-5 carbons such as propionate inhibit carbohydrate response element binding protein (ChREBP), acetyl-coenzyme A carboxylase (ACC), and fatty acid synthase (FAS) which plays role in liver lipogenesis.
Polyphenols showed anti-inflammatory effect through several signaling pathways, such as by suppressing the activation of the NF-κB pathway, decreasing JNK phosphorylation protein, reducing levels of serum inflammatory cytokines, and increasing antioxidant defenses via the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. In addition to polyphenols, other components in apples, such as pectin and its fermented products (SCFAs), can inhibit the secretion of TNF-α and NF-κB activation that suppress the progression of liver damage.

The ability of apple extract on improving histopathological features of fatty liver can also be seen from previous studies with similar results with this study. In addition, apple extract also showed lower levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG) compared to Western diet (high fat and sugar) group. Further research is needed to investigate certain active ingredient isolates in *Malus sylvestris* extract, which have dominant role on ameliorating NAFLD histopathological features. It is also necessary to examine the effects of *Malus sylvestris* extract other than histopathological studies, as the diagnosis of NAFLD may be made by other tests.

**Conclusion**

*Malus sylvestris* extract improve the degree of steatosis and portal inflammation histopathological features of hypercholesterolemic rat fatty liver. This shows that *Malus sylvestris* provide hepatoprotective effect that could act as a potential treatment for NAFLD.

**Conflict of Interest:** The authors declare no conflict of interest.

**Ethical Clearance:** This study had been approved by Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

**Source of Funding:** Self funding.

**Acknowledgements:** The authors are thankful to the authorities of Faculty of Medicine, Universitas Airlangga for providing the necessary facilities for this experiment.

**References**


Hyperglycemia in Childhood Acute Lymphoblastic Leukemia During Induction Chemotherapy

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Abstract

Background: Hyperglycemia is a recognized side effect of the corticosteroids and asparaginase given during induction chemotherapy for pediatric acute lymphoblastic leukemia (ALL). The ALL is the malignant tumor with the highest incidence in the childhood. The aim of this study is to investigate the impact of hyperglycemia during induction chemotherapy in childhood ALL.

Methods: This prospective study was done in Dr. Soetomo hospital from January to April 2018. The subject was newly diagnosed as ALL under the age of 18 years, treated with Indonesian childhood ALL 2013 protocol (Standard Risk (SR) group and High Risk (HR) group). Hyperglycemia was defined as at least two separate random plasma glucose levels > 200 mg/dL, which was evaluated before and during induction chemotherapy. Statistical analysis using Paired T-test for parametric and Wilcoxon Test for nonparametric.

Results: Thirty-three children were enrolled, 18/33 boys with mean age 5.8 (SD 3.78) years, compromised as ALL-L1 30/33. They were treated with ALL-HR 19/33 and ALL-SR 14/33. In overall groups, the mean random blood glucose level significantly increased from 108 (SD 21.3) mg/dL to 147 (SD 48.1) mg/dL, (mean difference 38.67 mg/dL; 95% CI 18.08 to 59.26 mg/dL, P=0.008). In SR group, there was a significant increased of mean random blood glucose level from 102 (SD 13.5) mg/dL to 133 (SD 37.3) mg/dL, (mean difference 31.8 mg/dL; 95% CI 8.78 to 54.8 mg/dL; P=0.01). In HR group, the mean random blood glucose level increased from 113 (SD 51.9) mg/dL to 165 (SD 25.4) mg/dL, (mean difference 51.9 mg/dL; 95% CI 18.6 to 85.2 mg/dL, P=0.004).

Conclusion: Blood glucose level is significantly increase during induction chemotherapy in both SR and HR

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Keyword: Hyperglycemia, acute lymphoblastic leukemia, childhood.

Background

Hyperglycemia is a common side effect of acute lymphoblastic leukemia (ALL) therapy. It has long
been recognized as a consequence of corticosteroids (either prednisone or dexamethasone) and asparaginase, chemotherapeutic agents key to ALL treatment. These medications are usually administered concurrently in high doses during the initial induction phase of chemotherapy. As a result, hyperglycemia frequently develops during this phase, with resolution after the steroids and asparaginase have been discontinued or reduced in dose.\(^1\^-4\) The potential causes may include beta cell dysfunction caused by chemotherapeutic drugs such as L-asparaginase, increased insulin resistance and hepatic gluconeogenesis induced by corticosteroids, or synergistic effects of these medications, given that these pharmacological agents are usually combined during initial induction therapy.\(^1\^-4\) The spectrum of hyperglycemia can range widely from transient isolated episodes to severe life-threatening complications such as diabetic ketoacidosis or nonketotic hyperglycemic hyperosmolar syndrome.\(^5\^-7\) Transient hyperglycemia developed during this period largely resolves as the chemotherapy is discontinued.\(^3\) However, affected children may need longer hospitalization and delay in chemotherapy; they may experience increased infective incidence and may even have poorer survival outcomes.\(^8\^-9\)

Previous studies have documented hyperglycemia induced by chemotherapy occur in 0.2-16%. Few published data have referred to the epidemiology of treatment-related hyperglycemia in Indonesian childhood ALL. The purpose of the current study is to evaluate the incidence of hyperglycemia during the induction chemotherapy for childhood ALL in Dr. Soetomo Hospital.

**Methods**

This is a prospective study involving of consecutive patients, age younger than 18 years, in whom ALL was diagnosed and who were admitted to Dr. Soetomo Hospital, Surabaya between January and April 2018.

We excluded patients with previously diagnosed diabetes mellitus (DM) or who were treated with glucocorticoids were also excluded. Medical records were reviewed to obtain relevant clinical data, including demographic information, such as age at diagnosis, sex, weight, height, and clinical parameters, such as initial white blood cell count (WBC), initial C-reactive protein (CRP) level, initial plasma glucose level, immunotyping of leukemic cells, and risk classification. Hyperglycemia was defined as at least two separate random plasma glucose levels ≥200 mg/dL according to the published guidelines for childhood diabetes.\(^10\) Plasma glucose level was evaluated before and during induction chemotherapy. BMI was calculated by dividing weight in kilograms by height in square meters. Using reference standards from the Centers for Disease Control and Prevention (CDC), each subject’s body mass index (BMI), BMI percentile for age, and BMI z-score for age were calculated. Per current CDC guidelines, subjects with BMI greater than or equal to the 95th percentile for age and gender were defined as overweight and those with BMI greater than or equal to the 85th percentile for age and gender were defined as at risk for overweight.\(^11\) All patients were treated according to the Indonesian childhood ALL 2013 protocol. The 1\(^{st}\) day of treatment was designated when the use of steroids was started. The entire procedure was approved by the Ethic Committee Dr. Soetomo Hospital, Surabaya.

All of the data collected were entered into an SPSS database (SPSS version 21.0.0.0). Descriptive statistics about the subject population were calculated, including data such as sex, age, and BMI at diagnosis. Presence of hyperglycemia was defined as above. Proportions of subjects with hyperglycemia overall and within each population category group (High risk and standard risk group) were calculated. For this analysis, subjects were divided by high risk and standard risk group as defined above. Chi-square analysis was used to determine the magnitude of difference in prevalence of hyperglycemia between groups. Statistical analysis using Paired T-test for parametric and Wilcoxon Test for non parametric. P values < 0.05 were regarded as significant.

**Results**

Thirty-three children were enrolled during our study period. All study subjects were newly diagnosed with ALL and received induction chemotherapy between January and April 2018. There were 18/33 boys and 15/33 were girls, the mean age of the patients when initially diagnosed was 5.8 (SD 3.78) years old. The patients compromised as ALL-L1 30/33 patients. They were treated with ALL-HR 19/33 patients and ALL-SR 14/33 patients. Baseline characteristics at the time of
testing are shown in Table 1.

In overall groups, 6/33 patients experienced hyperglycemia during induction chemotherapy, five of them treated with ALL-HR regimen and one treated with ALL-SR regimen. There is no significant incidence of hyperglycemia between ALL-HR and ALL-SR with p value 0.117 (Table 2). In overall groups, the mean random blood glucose level significantly increased from 108 (SD 21.3) mg/dL to 147 (SD 48.1) mg/dL, (mean difference 38.67 mg/dL; 95% CI 18.08 to 59.26 mg/dL, P=0.008).

**Table 1. The baseline characteristics of the patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (54.5)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (45.5)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;10 y</td>
<td>27 (81.8)</td>
</tr>
<tr>
<td>≥10 y</td>
<td>6 (18.2)</td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
<td></td>
</tr>
<tr>
<td>Undernourished</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>Well-nourished</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>Overweight</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Obese</td>
<td>0</td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 20mg/dL</td>
<td>27 (81.8)</td>
</tr>
<tr>
<td>≥20 mg/dL</td>
<td>6 (18.2)</td>
</tr>
<tr>
<td><strong>Risk group</strong></td>
<td></td>
</tr>
<tr>
<td>Standard risk</td>
<td>14 (42.4)</td>
</tr>
<tr>
<td>High risk</td>
<td>19 (57.6)</td>
</tr>
</tbody>
</table>

In overall groups, the mean random blood glucose level significantly increased from 108 (SD 21.3) mg/dL to 147 (SD 48.1) mg/dL, (mean difference 38.67 mg/dL; 95% CI 18.08 to 59.26 mg/dL, P=0.008). In SR group, there was a significant increased of mean random blood glucose level from 102 (SD 13.5) mg/dL to 133 (SD 37.3) mg/dL, (mean difference 31.8 mg/dL; 95% CI 8.78 to 54.8 mg/dL; P=0.01).
Table 2. Hyperglycemia in ALL HR and ALL SR

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Hyperglycemia</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>ALL HR</td>
<td>5</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>ALL SR</td>
<td>1</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>27</td>
<td>33</td>
</tr>
</tbody>
</table>

In HR group, the mean random blood glucose level increased from 113 (SD 51.9) mg/dL to 165 (SD 25.4) mg/dL, (mean difference 51.9 mg/dL; 95% CI 18.6 to 85.2 mg/dL, P=0.004), the increased blood glucose during induction chemotherapy are demonstrated in Table 3.

Table 3. Increased blood glucose during induction chemotherapy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before chemotherapy</th>
<th>During chemotherapy</th>
<th>Mean (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL HR</td>
<td>113 (SD 51.9)</td>
<td>165 (SD 25.4)</td>
<td>51.9 (18.6–85.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>ALL SR</td>
<td>102 (SD 13.5)</td>
<td>133 (SD 37.3)</td>
<td>31.8 (8.78–54.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Overall</td>
<td>108 (SD 21.3)</td>
<td>147 (SD 48.1)</td>
<td>38.67 (18.08–59.26)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Discussion

In this study, 33 children with newly diagnosis of acute lymphoblastic leukemia that treated in Dr Soetomo hospital and were in induction chemotherapy of their treatment, evaluated for hyperglycemia. These Children followed for six weeks and were assessed for growth parameters, and blood sugar. Hyperglycemia defined when at least two random glucose level >200 mg/dl. According to these criteria, there were six cases with hyperglycemia (18% of the subjects). In a study by Banihashem et al, 32 patients with a diagnosis of acute leukemia, and 17.2% of patients had hyperglycemia.\textsuperscript{12}

In this study, there was no significant difference between incidence of hyperglycemia and type of treatment according to Indonesia childhood ALL protocol 2013 (chi-square p=0.117). This result similar with Banihashem study, which is there was no significant difference between incidence of hyperglycemia and different protocol of chemotherapy that used for treatment of children (chi-square p=0.983).\textsuperscript{12} The similar study was a study by Baillargeon et al. that evaluated transient hyperglycemia in Hispanic children with Acute Lymphoblastic Leukemia, and 11.0% of the study cohort developed hyperglycemia during induction chemotherapy.\textsuperscript{4}

In this study, the blood glucose level is significantly increase in all groups during induction chemotherapy, overall with p value 0.008, p value 0.004 and 0.01 in HR groups and SR groups, respectively.
At a literature by Lowas et al. Prevalence of transient hyperglycemia during induction chemotherapy for pediatric acute lymphoblastic leukemia was assessed. Transient hyperglycemia (TH) is a recognized side effect of the corticosteroids and asparaginase given during induction chemotherapy for pediatric acute lymphoblastic leukemia (ALL). This study examined the prevalence of TH in a cohort of pediatric ALL patients and the impact on TH of type of steroid or asparaginase.

Hyperglycemia and diabetes induced by chemotherapy occur in the range of 0.2% to 16% (see table 4). Pui et al reported hyperglycemia in 9.7% of the pediatric ALL patients in the induction period of chemotherapy, after receiving prednisone and L-asparaginase. Weiser et al documented an incidence of hyperglycemia in 37% of patients during induction chemotherapy. According to Pastore et al 50% of ALL children may develop hyperglycemia, whereas Banihashem et al reported that 27.5% of the pediatric patients showed either diabetes mellitus or hyperglycemia.

There is a complex pathophysiology mechanism that explains the development of hyperglycemia in the pediatric population receiving induction chemotherapy. DeFronzo et al have described a series of events that lead to genesis of diabetes as follow: 1) some patients showed a predisposition to enhance insulin resistance and propensity for β-cells failure, 2) specific risk factors such as preexisting obesity reinforce these defects, 3) diabetes mellitus comes up, when a concomitant insulin secretory defect is present, regardless of the etiology.

During the standard treatment of ALL, children receive 3 drugs in the first month of the therapy. These include L-asparaginase, Vincristine and steroids as prednisolone. Children in high risk group of ALL receive a fourth chemotherapy drug, mostly daunorubicin. During the standard treatment of ALL, children receive 3 drugs in the first month of the therapy. Additionally, intrathecal chemotherapy using commonly methotrexate is performed. L-asparaginase inhibits insulin protein synthesis. L-asparaginase may directly reduce the glucose-stimulated release of insulin from β-cells and indirectly reduce insulin production by causing pancreatitis. Furthermore, it is known, that corticosteroids induce insulin resistance. These effects may lead to the development of diabetes mellitus in children receiving chemotherapy, and more often in those with additional risk factors.

Hyperglycemia occurs commonly in the pediatric population receiving induction chemotherapy, but a combination of glucocorticoids and L-asparaginase may cause diabetes mellitus. Physicians should be aware of these risk factors and perform an early and careful screening for hyperglycemia (fasting glucose levels) during the treatment of the patients with ALL.

Furthermore, application of the right treatment for hyperglycemia, such as insulin, permits an early clinical and biochemical normalization and enforces thereby the continuation of the chemotherapy. The use of insulin may reduce the period of hyperglycemia and therefore the possible future metabolic side effects.

**Conclusion**

The incidence of hyperglycemia is not significant in this study, but blood glucose level is significantly increase during induction chemotherapy in both SR and HR Indonesian childhood ALL 2013 protocol. Therefore, we recommend clinicians should be aware of the risk of hyperglycemia in childhood ALL during the induction chemotherapy. Future investigations of pediatric cohorts are needed to evaluate the influence and outcome of transient hyperglycemia as well as diabetes mellitus for long-term survival and metabolic syndrome.

**Conflict of Interest**: None declared

**Funding**: None declared

**Ethical Clearance**: Taken from Ethic Committee Dr. Soetomo Hospital, Surabaya.

**References**


11. Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion. CDC. 2000; 11; 13-4.


Correlation of Hot Work Climate with Dehydration on Bricks Workers

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Abstract
Each workplace must have its own source of danger which can cause health problems or occupational diseases due to the exception of informal sector workers such as brick workers. Health problems that can be experienced are dehydration caused by exposure to hot working climate. Incidence of dehydration can cause workers to get tired quickly and decrease productivity. The purpose of this study was to analyze the correlation between hot working climate with dehydration on bricks workers in one of Mojosulur’s brick home industries. Methods of his research is an analytic observational study, with cross sectional design. The subjects of this study were all brick workers in one of the brick home industries in Mojosulur by 30 respondents. The results of the study showed that most brick workers were dehydrated, varying in level, both moderate and severe dehydration. The results of statistical analysis show that the correlation coefficient (r = 0.638) which can be interpreted as a work climate factor has a strong correlation with the incidence of dehydration Conclusion of this research on work climate and dehydration of brick workers in one of Mojosulur’s brick home industries have a very strong correlation.

Keywords: bricks, dehydration, work climate, workplace,

Introduction
The progress of science and technology in Indonesia is currently taking place very rapidly, as is the process of industrialization of Indonesian society which is increasingly increasing. The development of industry in Indonesia is marked by the increasing number of small-scale industries that have sprung up both in the formal and informal sectors. But often the occupational safety and health of informal sector workers receive less attention, therefore there is a need for labor protection to be done to protect workers from work-related hazards 1.

The Central Statistics Agency in February 2018 released official statistics on employment in Indonesia, and based on the data released 58.22% or 73.98 million people worked in the informal sector and 41.78% were workers in the formal sector [2]. From the results these calculations show that in Indonesia more workers are working in the informal sector. One example of work in the informal sector includes the home industry making red bricks.

Each workplace must have its own source of danger which can cause health problems or occupational diseases due to the exception of informal sector workers such as brick workers. Based on research conducted by Wahyuni in 2016 on hazard analysis and the assessment of the need for personal protective equipment in brick workers in Demak, it was found several hazards, one of which was the danger of hot working climate and exposure to solar radiation caused by the presence of outdoor work climate and the presence of the process of burning bricks carried out in a long time 3. This makes dehydration and heat stress possible for workers.

Dehydration is a condition of lack of fluids in the body and can have an impact on the existing regulatory processes in the body 4. This is because there is exposure...
to hot temperatures at work followed by excessive sweating. Based on research conducted by THIRST (The Indonesian Regional Hydration Study) in Indonesia shows that many Indonesian people experience mild dehydration, especially in the group of adolescents aged 15-18 years and adults aged 25-55 years. That matter supported by research conducted by Hardinsyah which states that as many as 46.1% of Indonesian people experience mild dehydration, and among them are people who live in low-lying areas such as in Jakarta, Surabaya and Makassar.

In addition, Indonesian people’s water consumption is still relatively low, this is indicated by the results of research conducted by The Indonesian Regional Hydration Study (THIRST) which also shows that as many as 49.1% of research subjects experienced less water or mild hypovolemia, and in adults reached as many as 42.5%. Also reported that 43% of adults are in a state of dehydration at work.

The condition of dehydration that takes place continuously can cause a person experiencing muscle spasms. Fatigue in workers is also one of the effects caused by the occurrence of dehydration, someone who experiences fatigue makes the body become weak and lazy to do physical activity. If the condition is left and continues to increase the risk of kidney stone disease, urinary tract infections, cancer, colon, obesity constipation, cerebral vascular stroke and other health problems. The Other studies mentioning that if the body experiences a lack of fluids reaching more than 2% of the total fluids in the body will affect the quality of work done by the worker and if dehydration reaches 3.1% can reduce the quality of someone at work by 48%. In the study also mentioned that. If someone does not consume fluids during their activities for 6 hours can cause dehydration of 6.4% and reduce the quality of work by 25%.

Decreased cognitive performance, decreased physical and psychomotor endurance are also one of the effects of dehydration. Dehydration with weight loss of 1-2% has been shown to reduce short-term memory and the decline in cognitive function including short-term memory will cause a person’s difficulties in working and completing tasks.

Unnoticed dehydration can be experienced by people who perform physical activities exposed to heat and exposed to hot climate in the workplace. The working climate is the result of a combination of temperature, humidity, speed of air movement and heat radiation due to the level of heat expenditure from the body of the workforce as a result of his work. Weather or work climate that is not comfortable and not in accordance with labor standards and provisions that have been in force will result in a decrease in the capacity of workers, causing a decrease in work efficiency and productivity.

According to Tarkawa excessive hot work climate can cause fluid in the body to come out through sweat continuously and experience an increase can result in dehydration and other health problems. In addition, workers who carry out their work without realizing it can experience a decrease in fluid intake by losing a lot of body fluids such as sodium and this can lead to dehydration.

Regarding several studies Regarding the status of dehydrated workers. Research conducted by Sari shows the effect of heat work has a significant effect on the incidence of dehydration. This is indicated by the number of 66.67% or 10 respondents who experienced mild dehydration in the boiler section and the results of the Man Whitney test showed a related effect of heat work on dehydration (p = 0.023). Subsequent research conducted at PT Indo Acidatama Tbk, Kemiri showed that respondents who were dehydrated based on measurements of the concentration of urine color in hot work climates that exceeded the NAV were more numerous than the number of respondents who were not dehydrated. This is indicated by the results of the study that there are 6 workers who are dehydrated and 4 other respondents are not dehydrated.

Brick artisans are one example of industrial workers in the informal sector. Making bricks is one type of work that most of the activities carried out in the outdoor environment, it is possible for workers to be exposed to heat in each production process, especially in the process of grinding, drying, molding, structuring and burning. In addition to basic materials, the quality of bricks also depends on the combustion process carried out. The burning process that lasts for a long time can even take...
up to 1 day. During combustion it is necessary to have adequate heat and the flame must always be considered to produce good quality and not cause a fire, this results in workers being required to work watching the fire all day. Continuous exposure to heat from the sun at work, especially at milling, structuring, drying and burning processes can enable workers to become dehydrated, in addition to that found several symptoms in brick craftsmen such as dry skin, dry mouth and sometimes feel very thirsty. Based on these data, the researchers are interested in conducting research on the correlation of hot work climate with dehydration events.

**Methods**

Research carried out including observational research types, with cross sectional approach because it is carried out at a certain time. The location of data collection was carried out in one of Mojosulur’s brick home industries in Mojokerto Regency. The population of this study is all workers, amounting to 30 people.

In this study there are two variables in the independent variable, namely the work climate and the dependent variable dehydration events. Collecting work climate data using heat stress apparatus questemp 36. The measurement results will produce a wet ball temperature index (ISBB) that exceeds or is less than the NAV must be considered by looking at the workload of workers and working hours settings applied at the workplace. Then adjusted to the ISBB Table contained in Minister of Manpower Regulation 2018 Number 5 Year 2018. Dehydration in workers is measured by taking a urine specimen shortly after work and compared with a urine color indicator card by Armstrong 19. The measurement results are then recorded and categorized into 4 criteria: no dehydration, mild, moderate and severe dehydration in accordance with the card indicators listed. The more concentrated the color of urine the heavier the person is dehydrated. The questionnaire distributed was used to obtain supporting data about the characteristics of the respondents. Observations, interviews and secondary data used to find out about the general description of the Mojokerto Bricks industry.

The research data that has been obtained will then be analyzed by statistical tests to determine the relationship between variables. In this study there are 2 variables: work climate as an independent variable and dehydration as the dependent variable. Based on the objectives of the research conducted, this study uses the Spearman correlation test. The test is used to determine the strength of a relationship between variables by analyzing the value (r) of the correlation coefficient, so that it can be determined the strength of the relationship between the work climate and the dehydration status of workers.

**Findings**

**Overview Bricks Home Industry Mojosulur**

Home Industry Mojosulur Bricks is one of the small industries that produce red bricks that have been established since 2002. Home Industry Mojosulur Bricks. This Home Industry manufactures brick presses labeled MRH and still uses traditional methods. This Home Industry is located on Jl. Teuku Umar RT 01 / RW 07 Mojosulur Village, Mojosari District, Mojokerto. In the process of making red bricks in the home industry entirely using human labor from the stage of processing land and raw materials to the finishing process. There are 5 stages in the process of making bricks including the process, mixing materials, grinding, molding, drying and burning bricks. The bricks produced by this home industry have several advantages, which are high density, more economical, durable and have good adhesion and are suitable for all soil textures.

**Identification of Respondent Characteristics**

Identification of Respondent Characteristics. The results of the questionnaire showed that there were 5 people (16.7%) who were aged in the 26-35 years age group. There were 10 people (33.3%) who were around 36-45 years old, there were 12 people (40%) who were around 46-55 years old, and 3 people (10%) were in the 56-65 years age group. The age of workers in the Mojosulur brick industry is the most workers in the age group of 46-55 years with a total of 10 people (33.3%). The results of the questionnaire based on gender showed that in the brick home industry there were more male workers as many as 19 people (63.3%) and 11 people (36.7%) were female workers. Based on the results of interviews with respondents as many as 20 people (66.7%) have worked > 2 years and 10 people (33.3%) worked ≤ 2 years.
The Frequency Distribution of Work Climate

The measurement of the working climate is done by using the Temp Temp 36 tool and the measurement is done at 5 points, namely at each stage of the brick making process, Point A is located at the mixing point of the brick material. Point B is located in the brick material mill. Point C is located at the place where the brick material is placed, Point D is located at the brick drying area and point E is located at the brick kiln.

Table 1. Work Climate Mesurement on the Mojosulur Home Bricks Industry 2020

<table>
<thead>
<tr>
<th></th>
<th>Sb (oC)</th>
<th>Sk (oC)</th>
<th>Sg (oC)</th>
<th>ISBB (oC)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material Mixing</td>
<td>27,2</td>
<td>31,4</td>
<td>43,6</td>
<td>31,1</td>
<td>61</td>
</tr>
<tr>
<td>Material Milling</td>
<td>27,8</td>
<td>33,0</td>
<td>45,2</td>
<td>31,8</td>
<td>43</td>
</tr>
<tr>
<td>Material Forming</td>
<td>26,2</td>
<td>31,6</td>
<td>33,5</td>
<td>28,4</td>
<td>58</td>
</tr>
<tr>
<td>Bricks Drying</td>
<td>27,8</td>
<td>32,5</td>
<td>43,4</td>
<td>31,4</td>
<td>49</td>
</tr>
<tr>
<td>Bricks Burning</td>
<td>26,9</td>
<td>32,9</td>
<td>48,9</td>
<td>31,9</td>
<td>47</td>
</tr>
</tbody>
</table>

From the measurement results on Table 1, it can be seen that the highest ISBB is at point E where the brick kiln process is 31.9 °C. This is due to the additional heat from the brick kiln and the lowest ISBB is 28.4 °C at point C, which is located at the site the process of laying bricks which is indeed a workplace is an indoor workplace. Setting working hours in the brick brick home industry is an average of 50-75%. Thus the NAV for hot working climate is 31.0 °C in the category of light workloads, 29.0 °C for workers with moderate workloads, and 27.5 °C for workers with workloads hard.

Table 2. The Frequency Distribution of Work Climate (NAV)

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤NAV</td>
<td>10</td>
<td>33,3</td>
</tr>
<tr>
<td>&gt;NAV</td>
<td>20</td>
<td>66,7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100,0</td>
</tr>
</tbody>
</table>

From table 2, It can be seen that the results of ISBB NAV determination in Mojosulur brick home industry are 20 respondents (66.7%) who are exposed to heat > NAV and 10 respondents (33.3%) are exposed to heat temperature ≤NAV.

The Frequency Distribution of Dehydration

Dehydration measurement is done when the worker has finished his work, and will move home from work. Dehydration measurement is done by asking the worker urine sample and seeing the urine color density which is then compared with the urine color indicator which has 8 colors and then categorized into 4 categories not dehydrated, mild, moderate and severe dehydration. Following are the results of the distribution of respondents based on the measurement of dehydration level showed on table 3.

Table 3. The Frequency Distribution of Dehydration

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Dehydration</td>
<td>7</td>
<td>23,3</td>
</tr>
<tr>
<td>Mild</td>
<td>7</td>
<td>23,3</td>
</tr>
<tr>
<td>Moderate</td>
<td>8</td>
<td>26,7</td>
</tr>
<tr>
<td>Severe</td>
<td>5</td>
<td>16,7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100,0</td>
</tr>
</tbody>
</table>

From the results of dehydration measurements in table 3 shows that as many as 7 people (23.3%) experienced mild dehydration, 8 people (26.7%) experienced moderate dehydration, 5 people (16.7%) experienced severe dehydration. This shows that the highest number of brick craftsmen workers is moderate dehydration, namely as many as 8 people (26.7%)
**Correlation of work climate with dehydration**

Based on the work climate measurement results are grouped into 2 namely for setting working time 50-75 ≤ NAV (For light workloads ≤ 31.0 °C, moderate workloads ≤ 29.0 °C, and heavy workloads ≤ 27.5 °C) and> NAV (For light workloads> 31.0 °C, moderate workloads> 29.0 °C, and heavy workloads> 27.5 °C) While work dehydration status is categorized into 4 categories namely no dehydration, dehydration mild, moderate dehydration, and severe dehydration. Below is a cross tabulation between the hot work climate and the dehydration of Mojosulur’s brick home industry workers showed on table 4.

<table>
<thead>
<tr>
<th>Work Climate</th>
<th>Dehydration Level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Mild</td>
</tr>
<tr>
<td>≤NAB</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>&gt;NAB</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>30</td>
</tr>
</tbody>
</table>

Based on the table above shows that in the measurement of hot work climate ≤ NAV many workers experienced mild work dehydration, namely as many as 3 workers with a percentage of 10%. Then in the measurement of heat stress> NAV experienced the most moderate dehydration as many as 7 workers with a percentage of 23.3%.

**Table 5. Correlation Between Work Climate And Dehydration**

<table>
<thead>
<tr>
<th>Work Climate</th>
<th>Dehydration</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value Spearman Correlation</td>
<td>0.638</td>
<td>Very Strong</td>
</tr>
</tbody>
</table>

Analysis conducted using statistical calculations, based on the Spearman correlation test that has been done, the results obtained correlation coefficient (r) = 0.638. These results indicate the resulting correlation coefficient approaching 1 which means that there is a strong relationship between the hot work climate with the incidence of dehydration in home brick workers in Mojosulur, Mojokerto Regency.
Discussion

Work Climate

In the Heat Stress measurement using the ISBB (Ball and Wet Temperature Index) method using the Questemp 36 at 5 points in accordance with the worker’s job. The lowest Ball and Wet Temperature Index results are obtained at 28.4°C at Point C, where the brick laying process is included in the indoor work area and the highest Ball and Wet Temperature Index at 31.9°C is at point E, at the outdoor work area. who worked on the brick burning process. According to Permenakertrans No. 5 of 2018 concerning NAV (Threshold Value) Physical and Chemical Factors in the Workplace, the NAV of the working climate is determined by measurement, workload and also work time. The working time in the brick brick home industry in Mojosulur is 50-75% due to workers not working full hours 8 hours a day. So for NAV that is in accordance with the workplace conditions in the brick brick home industry for light workloads of 31.0°C, medium workloads of 29.0°C, and heavy workloads of 27.5°C. Therefore, it can be concluded that the most measurement is> NAV of 20 people (66.7%).

The temperature at work can be influenced by work processes and work environment factors themselves. As long as the worker’s body is active, the body will directly try to keep up with the temperature in the environment and try to balance the heat temperature environment received by the body with the heat in the body through the process of heat loss in the body. A comfortable temperature for Indonesians at work is between 24-26°C. A hot work climate can cause heat stress to be received by workers who are working in the workplace environment as an additional heat load (in addition to the heat load generated by the body which can result in many negative effects on workers in the form of work disorders or health problems such as dehydration, increased pulse, dizzy headaches, dizzy eyes, rapid stomach nausea Tired. When workers are exposed to hot temperatures it will reduce agility, extend reaction time and slow down decision-making time, interfere with brain work accuracy, interfere with coordination motor sensory nerves, and make it easier for emotions to be stimulated.

Dehydration

Dehydration is a condition in which the lack of fluids in the body due to the amount of fluid that exits is greater than the amount of fluid that enters. If the body loses a lot of fluids, the body will become dehydrated. Measurement of dehydration work done on brick workers in the Mojosulur brick home industry was carried out by the observation method through observing the color of the workers’ urine, then categorized as not dehydrating, mild dehydration, moderate dehydration and severe dehydration. Then the measurement results obtained are 7 people (23.3%) not dehydrated status, 7 people (23.3%) mild work dehydration status, 8 people (26.7%) moderate dehydration status, and severe dehydration status 5 people (16.7%).

A worker who is exposed to heat and without using proper personal protective equipment will be disturbed by his endurance. The body will neutralize body heat by sweating which results in reduced fluid and electrolyte salts in the body. Consumption of adequate drinking water can reduce the effects of dehydration due to heat pressure. This is because in drinking water there are electrolyte salts in the form of cations and anions needed by the body. The main cations contained in drinking water are sodium (Na+) and Potassium (K+) while the main anions are chloride (Cl-). One of the dangers of brickworkers’ workplaces is hot workplace temperatures due to direct exposure to sunlight and the additional heat from the combustion process so as to accelerate the dehydration experienced by workers. Therefore there is a need for control by home industry owners so that work dehydration can be reduced so that there is no decrease in work productivity.

Relationship of Work Climate with Dehydration

According to the Regulation of the Minister of Manpower and Transmigration of the Republic of Indonesia Number 5 Year 2018 Regarding NAV Physical and Chemical Factors in the Workplace, the NAV of the working climate with a working time of 50% - 75% ie 31°C for light workloads, 29°C for moderate workloads and 27.5°C for heavy workloads. Based on work climate measurements that have been made on the Mojosulur Bata Home Industry workers there are 20 respondents (66.7%) who work in working climate conditions> NAV and 10 respondents (33.3%) who
work in working climate conditions ≤ NAV. Workers who work in working climate conditions > NAV are more dehydrated, compared to workers who work in working climate conditions ≤ NAV. In working climate conditions > NAV

Using statistical calculations, based on the spearmen correlation test, the result \( r = 0.638 \) shows that there is a strong correlation between hot working climate and dehydration events. This is in line with the results of research conducted by Megayani Puspita Sari. The results of the analysis obtained information on the value of Pearson chi square with sig 0.00 < 0.05 so that Ho was rejected that’s mean there have correlation between hot working climate and dehydration.

In addition there are other studies that are in line with the results of this study, namely research conducted by Nensi which shows that there is a correlation between environmental physical conditions, namely between temperature and humidity at work with dehydration levels with the results of the value of the temperature contingency coefficient and dehydration level of 0.603 which means that the correlation between temperature and dehydration is very strong.

According to Suma’mur, workers who work in a workplace that exceeds the NAV of the working climate can experience the effects of heat stress. The effect of heat stress occurs as a result of the body’s process of maintaining body heat to no avail. The effect of heat stress can be in the form of subjective complaints due to heat stress such as complaining of heat, lots of sweat, always thirsty, feeling bad and loss of appetite caused by loss of fluid from the body by evaporation of sweat. Exposure to a physical work environment such as a hot work environment that continues can lead to health problems, one of which is dehydration.

**Conclusion**

Conclusion Based on the results of research conducted showed a strong correlation between the work climate with the dehydration incident experienced by workers at the Mojosulur Brick Industry, Mojokerto Regency. Most workers in the Mojosulur brick home industry are dehydrated in the moderate category, and only 7 workers are not dehydrated. Suggestions for home brick industry owners need to provide additional protection in a workplace that has a work climate that exceeds the Threshold Value so workers are not exposed to direct sunlight and the hot work climate can be reduced to less than the Threshold Value and needs to provide some a place for workers to take their drinks close to their work area so that when workers are feeling thirsty or dehydrated they can easily take the drinking water and drinks can be added with electrolyte salt.

**Conflict of Interest:** None.

**Source of Funding:** Self

**Ethical Clearance:** Ethical clearance was obtained from the Ethics Committee of the Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia

**References**


Equality of Suffrage for People with Mental Disorders in Malang City

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Abstract

Constitutional Court of Indonesia in 2015 established Constitutional Court’s Decision Number 135 / PUU-XIII / 2015 that revoked the provisions of Article 57 paragraph 3 letter a of Indonesian Law Number 8 of 2015 concerning Amendment of Law Number 1 concerning the Stipulation of Government Regulation in Lieu of Law Number 1 of 2014 which the substance prohibited person with disabilities for voting in election. Then, Law Number 7 of 2017 concerning General Elections, which exclusively gave political rights for people with disabilities to implement their political rights.

Qualifications for people with mental disabilities in elections of The Constitutional Court decides that the phrase “mental disorders/ memory impairment” must be interpreted as “experiencing mental illness and/ or permanent memory impairment which according to mental health professionals, has abolished one’s ability to vote in elections”. It means that sufferers with mental disorders and/ or impermanent memory impairment must still be registered as voters and they have the opportunity to use their voting rights in elections.

Equality of political rights for people with mental disorders (Orang Dengan Gangguan Jiwa (ODGJ)) in elections is very important because general election gives opportunity to increase participation and change public perception for the ability of people with disabilities. As the result, people with disabilities can have stronger political voice and they are recognized more as equal citizens. Moreover, this research was in category of normative research with normative juridical approach.

In conclusion, the equality of political rights for people with mental disorders (Orang Dengan Gangguan Jiwa (ODGJ)) in elections is very important because general election gives opportunity to increase participation and change public perception for the ability of people with disabilities. As the result, people with disabilities can have stronger political voice and they are recognized more as equal citizens.

Keywords: Voting Rights (suffrage), People with Disabilities, General Election

Introduction

Democracy in Indonesia is different from Western democracy. Western democracy is liberal or free democracy. Democracy in Indonesia is built from Pancasila, as a fundamental norm that is explained in law and regulation norms. Disabilities are part of Indonesian citizens who have rights for respect, protection, and fulfillment of their basic rights as what is guaranteed in the 1945 Constitution of the Republic of Indonesia. In legislative and presidential and vice presidential elections in 2014, people with disabilities still experienced discriminations and one of them was discrimination in using their right to vote in general elections. The cause was the absence of regulations that protected rights for people with disabilities. According to Abdul Rasyid Thalib, there are two authorities in Constitutional Court’s main authority, which are authority in examining law against the Constitution of the Republic of Indonesia and authority in interrupting

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on authority dispute of state institutions. \(^{(2)}\)

In 2015, the Constitutional Court determined Constitutional Court (Mahkamah Konstitusi (MK)) Decision Number 135 / PUU-XIII / 2015 that revoked the provisions of Article 57 paragraph 3 letter a of Indonesian Law Number 8 of 2015 concerning Amendment for Law Number 1 concerning the Stipulation of Government Regulation in Lieu of Law Number 1 of 2014, which the substance prohibited people with disabilities to vote in general election. Based on this Decision, people with disabilities obtain political justice and it refers to this Decision. The Government enacted Law Number 7 of 2017 concerning General Elections, which exclusively gave political rights for people with disabilities to use their political rights. In Article 5 of Indonesian Law Number 7 of 2017 stated that: “Person with disabilities who qualifies have same opportunity as Voter, as candidate for House of Representative, as candidate for Regional Representative Board, as candidate for President/ Vice President, as candidate for Regional House of Representative, and as Election Organizer”. \(^{(3)}\) The provisions of Article 5 of Law Number 7 of 2017 were in accordance with the provisions of Article 75 paragraph (2) of Law Number 8 of 2016 concerning Person with Disabilities which stated that, “Central Government and Regional Government must guarantee the rights and opportunities for Person with Disabilities to elect and to be elected”. Discriminatory provision in provision of Article 57 paragraph (3) letter a of Law number 8 of 2015 was submitted to the Constitutional Court (Mahkamah Konstitusi (MK)) of Indonesia to be conducted material test against provisions in the 1945 Constitution of the Republic of Indonesia, especially against Article 28D paragraph (1). Petition of material test was submitted on 20th October 2015. Then, on 27th September 2016, the Constitutional Court issued Decision Number 135 / PUU-XIII / 2015 against the petition. In Decision of amar (verdict), it was conveyed that the Court granted some petitioners, especially in stating that Article 57 paragraph (3) letter a of Law Number 8 of 2015 did not have binding legal force as the phrase of “mental disorders/ memory impairment” is not interpreted as “experiencing mental disorder and/or permanent memory impairment which according to mental health professionals, has abolished someone’s ability to vote in general elections”.

The Constitutional Court’s decision views person with disabilities in general, but what is being debated today is the participation of the person with mental disabilities in general elections as voter, which is called as People with Mental Disorders (Orang Dengan Gangguan Jiwa (ODGJ)). General Election Commission (Komisi Pemilihan Umum (KPU)) of Indonesia had stipulated Election Commission Regulation Number 11 of 2018 concerning the Compilation of Voter Lists which stipulated to allow people with mental disabilities to use their suffrage. General Election Commission (KPU) had included people with disabilities (ODGJ) in Permanent Voter List (Daftar Pemilih Tetap (DPT)) in general election in 2019. Around 5,000 people with mental disabilities had been registered in Permanent Voter List (Daftar Pemilih Tetap (DPT)). Although General Election Commission only included voters who qualified administrative requirements on the voter list, as long as the people with disabilities qualified the requirements, they must be given their suffrage. \(^{(4)}\)

However, being allowed for the people with disabilities (ODGJ) to vote in general elections caused procontra in legal experts, politicians, educators, students, communities, and all groups of community because there were fears of ODGJ vote which could be misused later by irresponsible elements. Some people believed that ODGJ could not vote. This opinion was based on the provisions of Article 433 of the Civil Code which stated that people with mental disorders must be with tutelage. Thus indirectly, ODGJ could not vote. According to Civil Code, person who is under control by tutelage is considered incapable in doing legal actions. In other words, the person with disabilities cannot be accountable for his/her actions. According to the provisions of Article 1330 paragraph (2) of Civil Code, person who is under tutelage is stated that he/she is not capable in law, such as a crazy person or memory loss person. \(^{(5)}\) All legal actions for people who are under the tutelage are represented by their tutelage as what is stipulated in the provisions of HIR Article 145 and Article 171 of Law 8/1981 concerning Criminal Procedure Law. Article 145 of HIR stipulates that one of the people who cannot be heard in court as witnesses is crazy people, even though the crazy people sometimes had bright memories. Thus, it might be understood that ODGJ is not capable in law.
**Method**

**Research Type**

Type of this research was empirical juridical research. In this research, the researcher examined the equality of political rights for people with mental disorders (orang dengan gangguan jiwa (ODGJ)) in regional elections in Malang City.

**Research Approach**

Type of this research approach was sociological juridical approach. Juridical approach in this research aimed at analyzing Indonesian Law Number 7 of 2017 concerning Elections, then, sociologically looking at the implementation of these regulations in Malang City people who were as participants in the election of Regional Government.

**Data Source**

- **Primary Data Source**

  Data was obtained from parties which were related directly with this research and the parties were:
  1. Batu City people
  2. Batu City people who became part/ who suffered from mental disorders.

- **Secondary Data Source**

  Supporting data were such as books, archives, documents, and many more which were obtained during research process.

**Technique of Data Collection**

- **Primary Data**

  Techniques which were used to collect primary data were observation, documentation, and question and answer verbally and directly which were open, dialogic, and systematic through in-depth interviews in order to explore more closely regarding the equality of suffrage for people with mental disorders in Batu City.

- **Secondary Data**

  Data was obtained through literature research which was collecting and studying literature in accordance with this research as a comparison of literature and theory studies.

**Discussion**

Mental disorders are indicated by person’s inability to assess reality. It is caused by a disturbance of neurochemical balance in nerve cells in human brain that makes the person is disrupted and unproductive in society. In Indonesia, implementation of human rights is guaranteed by the 1945 Constitution of Republic of Indonesia as well as Law Number 39 of 1999 concerning Human Rights, specifically in article 23 which states that everyone (including people with mental disorders) has rights to elect and political beliefs. In 2005, Indonesia also had ratified International Convention on Civil and Political Right (ICCPR), and had become an active legislation that was Indonesian Law Number 12 of 2005 in article 25 (b) which stated that every citizen had right and opportunity to elect and to be elected in honest periodic general elections with universal and equal suffrage.

In Indonesian Law Number 18 of 2014 concerning Mental Health, people who suffer from mental disorders/ memory impairment are called as People with Mental Disorders (Orang Dengan Gangguan Jiwa (ODGJ)) and determination (diagnosis) as ODGJ can only be conducted by certain professionals such as Psychiatrists, Doctors, and Clinical Psychologists. The diagnosis must be referred to Guidelines for the Classification of Mental Disorders in Edition III (PPDGJ III).

Clinically, in order to assess whether someone is considered to have capacity to make choice or not, at least, he/she must be able to state 4 things, which are understanding the choice that is given, being able to state their choice, having reasons why to choose the choice, and knowing the consequences from that choice. This capacity must be checked specifically for specific purposes or situations. The example of clinical situation is sufferer’s capacity needs to be examined in order to determine whether the sufferer has capacity to determine treatment type that will be given, or to determine his/her willingness to participate for the research. Of course, the sufferer must be given a complete explanation until he/she understands what the consequences from the treatment or the consequences from the participation in the research. The capacity in making choice aims...
to guarantee the choice that will be taken is the best choice for him/her and at the same time, it protects him/her from bad consequences that may arise. No one knows more about him/her, except for himself/herself. Therefore, the best choice for him/her can be different from ordinary people.

Psychosis people with mental disorders (Orang dengan Gangguan Jiwa (ODGJ)) can still function normally for most of his/her life. Generally, psychotic mental disorders are chronic and episodic (recurrent). In ‘relapse’ condition, the ODGJ experiences hallucinations, has wrong thoughts, or behaves improperly.\(^\text{(10)}\) The content of his/her thought becomes difficult to be understood, even his/her ideas and actions are specific only for him/her. Sometimes, sufferers can be difficult to be directed and they can behave uncooperatively.\(^\text{(11)}\) In serious clinical condition like this, the sufferers must get treatment without being asked about his/her willingness. Sufferers are considered not to have capacity to determine the treatment. If the period of this severe relapse is occurred on Election Day, it is certainly not possible to force sufferers to come to the voting place to participate in voting. However, out of the relapse period, the sufferer’s thoughts, attitudes, memories, and behavior can be normal.\(^\text{(12)}\) Regarding registration process for the voter, which until election day is begun for quite long period of time (3-6 months), removing someone from the voter list will remove the sufferer’s rights which on election day, he/she is most likely to be in good condition and able to vote.\(^\text{(13)}\)

**Conclusion**

Voting in elections is not a difficult thing. There are no wrong choices, which have bad consequences either for sufferer or for society. Each person’s choice is very personal and cannot be accused or blamed. A person is not determined by diagnosis or symptoms which are experienced by the sufferers, but it is from cognitive ability. It means that psychosis people with mental disorders (Orang dengan Gangguan Jiwa (ODGJ)) such as schizophrenics, bipolar, or severe depression do not automatically lose the capacity to make choice. The capacity can be reduced or considered to have no capacity for only sufferers who suffer from serious cognitive dysfunction. However, it needs to be known that cognitive function can be improved by learning and training. A person who is deemed not to have capacity can be educated and trained repeatedly, thus, his/her capacity can

\textbf{Conflict of Interest} : No

\textbf{Source of Funding} : Authors

\textbf{Ethical Clearance} : Yes.

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Determining the Exposure of Benzene, Toluene, Xylene (in Condensate) in a Chemical Laboratory of Natural Gas Company by Chemical Health Risk Assessment (CHRA)

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Abstract

Introduction: Chemical risk assessment is needed to find out how high the hazard level of chemicals in the laboratory. This chemical health risk assessment will assess how often the chemical is exposed to workers, how much exposure it is so that the level of risk can be understood. The purpose of enabling decisions to be made on appropriate control measure, training of employees, monitoring and surveillance activities as may be required to protect the health of employees who may exposed by hazardous chemicals to health at work.

Methods: This research is research with a qualitative approach. Based on the data collected, this study is observational. This study uses the Chemical Health Risk Assessment (CHRA) method. This method is carried out by identifying chemicals, determining Hazard Rating, evaluating exposure, assessing the adequacy of the control measures that have been carried out, concluding the CHRA results, and identifying the efforts that need to be made.

Result: In this study, there are three work units, namely a wet laboratory, oil laboratory, and gas laboratory. The chemicals analyzed are benzene, toluene, and xylene in the condensate. The hazardous chemical have significant risk now and already adequately controlled could increase in future (C2) which mean that the employees are at risk due to hazardous chemicals and it adequately controlled but the risk can increase in the future.

Conclusion: Therefore, several steps can be taken to control the risk of health hazards from benzene, toluene, and xylene in the condensate, for example: determine precaution to maintain controls and minimize chances of higher exposure occurring; determine additional measures for regaining control if a high-risk event occurs despite precaution; identify measures, procedures, and equipment to prevent or control any accidental emission of chemical hazardous to health, determine if monitoring or health surveillance is required to check on the effectiveness of controls; and review assessment every five years or when is a change in circumstances.

Key words: Hazardous chemicals, risk assessment, BTX, CHRA

Introduction

Natural gas processing companies are one of the industries that have laboratory facilities to support their production. However, the laboratory is one place where various chemicals gather in one place. Chemical exposure can have the potential to cause health problems for workers. Chemical laboratory workers have a significant risk of exposure to hazardous chemicals1.
inhale toluene can cause euphoria, drunkenness, dizziness, trembling, convulsions, respiratory disorders, and tremors. Through ingestion, toluene can cause oropharyngeal and digestive tract. On the eyes, it can cause lacrimation and corneal damage, while it may cause erythema and dry skin through dermal exposure. Effects due to chronic exposure to toluene via inhalation are damage to the liver, kidneys, and nerves, while to the skin it may cause contact dermatitis and irritation. Refinery natural gas workers are exposed to significant levels of benzene, toluene, ethylbenzene, and xylene (BTEX)\(^4\).

Chemical risk assessment is needed to find out how high the hazard level of chemicals in the laboratory. To carry out this risk assessment, all chemicals used must be identified, evaluated, and controlled against the hazards of chemicals that exist. This risk assessment is carried out as an effort to prevent health problems that may arise from exposure to chemicals. This chemical health risk assessment will assess how often the chemical is exposed to workers, how much exposure it is so that the level of risk can be understood.

**Material and Method**

This research is research with a qualitative approach. Based on the data collected, this study is observational. This study uses the Chemical Health Risk Assessment (CHRA) method. This method is carried out by identifying chemicals, determining Hazard Rating, evaluating exposure, assessing the adequacy of the control measures that have been carried out, concluding the CHRA results, and identifying the efforts that need to be made\(^5\).

The procedure in carrying out a DOSH, 2000 Chemical Health Risk Assessment (CHRA) is consists of ten aspects below:

**Chemical Identification and Hazard Rating (HR) Assessment**

The results of chemical identification are used to classify chemicals based on the health impacts that each chemical can cause. The grouping results will be assessed using the Hazard Rating (HR). Hazard Rating has a rating scale of 1 to 5, with the understanding that the rating scale of 1 is a scale for chemicals that are not dangerous to the health of the workforce, up to a rating scale of 5 which is a scale for chemicals that are very dangerous to the health of the workforce. Hazard Rating in the analysis of health risks due to chemicals is used to determine priorities based on the potential health hazards caused by chemicals.

**Duration of Exposure (Duration Rating / DR)**

Identifying the duration of exposure is done to assess the effects of chronic or routine exposure to the chemical used. Chronic effects assessments can use the total duration of exposure. The total duration of exposure is the product of the number of exposures in one week and the average duration of each exposure.

**Degree of Release or Occurrence of Chemicals**

The degree of release or appearance of chemicals in the environment can be estimated from information, including the physicochemical information contained in the Chemical Material Safety Data Sheet, the characteristics of the work process in the description of the chemical process, the number of chemicals used, the method of chemical use, environmental conditions workplace.

**Degree of Absorption or Contact with Chemicals**

Chemicals that are absorbed through the skin include organic solvents and some pesticides. The assessment of the degree of absorption or contact with chemicals must be based on the results of observations on the chemical that has the highest degree.

**Magnitude Rating (MR)**

Qualitatively, the Magnitude Rate or the amount of exposure is assessed based on the estimated dose of chemicals that are absorbed through inhalation and absorption on the skin. The absorption of chemicals through the eyes and skin comes not only from direct contact with chemicals but also from contaminated air, smoke, or particulates.

**Exposure Rating (ER)**

Exposure Rating (ER) is assessed through cross-tabulation based on the duration of exposure or Duration Rating (DR) and the amount of exposure or Magnitude Rating.
Assessment of the Adequacy of Controls That Has Been Done

Assessment of the adequacy of the control measures are evaluated for each work unit. This assessment is carried out simultaneously with the assessment of chemical exposure. The adequacy of the control that has been carried out is assessed by examining the control measures that have been made, checking the air inspection records, biological monitoring, and checking the inspection records, as well as testing the control equipment used.

Risk Rating (RR)

Determination of the risk level or Risk Rating is used to conclude the results of the chemical risk assessment that has been carried out. The risk level assessment is carried out based on the results of the exposure level or Exposure Rating and Hazard Rating that have been done. The level of risk will be evaluated based on “significant” and “insignificant”. The risk that is evaluated is “insignificant” if occupational exposure cannot cause health problems in the workforce.

Conclusion of Risk Assessment Results

The conclusion of the chemical risk assessment results can be made after the Risk Rating has been assessed. Based on the results of the Risk Rating assessment and assessment of existing control measures, there are 4 types of conclusions that can be reached in this assessment. The four types of conclusions are denoted by C1, C2, C3, C4, and C5.

Identification of Control Measures That Can Be Taken

The control measures that can be taken are identified based on the results of risk decisions resulting from the risk assessment that has been carried out. Some of these control efforts include:

a. Take appropriate measurements to control exposures that exceed safe limits.

b. Take measurements to eliminate intolerable risks.

c. End the assessment and set a new schedule to re-evaluate or review the assessment that has been done.

d. Decide whether exposure monitoring or health surveillance is more necessary.

e. Create a strategic long-term plan to control exposure to as low as reasonably practicable (ALARP).

f. Gather information or advice from specialists on a specific problem.

g. Maintain control equipment to keep working properly to implement a preventive maintenance program.

Result

In this study, there are three work units, namely a wet laboratory, oil laboratory, and gas laboratory. The chemicals analyzed are benzene, toluene, and xylene in the condensate. In this assessment, the chemical hazard will identify the type of chemical, the degree of exposure, and the adequacy of existing control measures. Based on these parameters a conclusion can be formed so that it can be used as input for efforts to reduce the risk of hazardous chemicals. The results of the following qualitative assessments obtained from the MSDS are shown in tables 1, 2, 3, and 4 below.

<table>
<thead>
<tr>
<th>No.</th>
<th>Work unit</th>
<th>Duration of Work</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wet Laboratory</td>
<td>7am - 5pm</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Oil Laboratory</td>
<td>7am - 5pm</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Gas Laboratory</td>
<td>7am - 5pm</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2. Hazard Rating Determination of Benzene, Toluene, and Xylene in Condensate

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Chemical</th>
<th>Determine the Degree of Hazard</th>
<th></th>
<th>Hazard Category</th>
<th>HR</th>
<th>Effect</th>
<th>Route of Exposure</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene (in condensate)</td>
<td>Category 1 carcinogens,</td>
<td>5</td>
<td>HR</td>
<td></td>
<td>Carcinogen and mutagen</td>
<td>R45 / R46</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mutagens, and teratogens</td>
<td></td>
<td>Hazard Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Toluene (in condensate)</td>
<td>Skin irritation</td>
<td>2</td>
<td>HR</td>
<td></td>
<td>Toxic and harmful</td>
<td>R48 / R20</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Xylene (in condensate)</td>
<td>Skin Irritation</td>
<td>2</td>
<td>HR</td>
<td></td>
<td>Harmful</td>
<td>R20 / R21</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3. Exposure Rating of Determination of Benzene, Toluene, and Xylene in Condensate

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Chemical</th>
<th>Evaluate Exposure</th>
<th></th>
<th>Degree of Release</th>
<th>Degree of Absorption</th>
<th>Magnitude Rating</th>
<th>Exposure Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene (in condensate)</td>
<td></td>
<td>4</td>
<td>High</td>
<td>Moderate</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Toluene (in condensate)</td>
<td></td>
<td>4</td>
<td>High</td>
<td>Moderate</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Xylene (in condensate)</td>
<td></td>
<td>4</td>
<td>High</td>
<td>Moderate</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Discussion

Table 4. Concluding the Assessment of Benzene, Toluene, and Xylene in Condensate

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Chemical</th>
<th>Determining Degree of Hazard</th>
<th>Determining Degree of Exposure</th>
<th>Risk Rating</th>
<th>Risk Evaluation</th>
<th>Adequacy of Control Measure</th>
<th>Conclusion of Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene (in condensate)</td>
<td>Hazard Rating (HR)</td>
<td>Exposure Rating (ER)</td>
<td>5</td>
<td>4</td>
<td>5 Risk Significant-Category 2</td>
<td>Adequate</td>
</tr>
<tr>
<td>2</td>
<td>Toluene (in condensate)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>Risk Significant-Category 1</td>
<td>Adequate</td>
<td>C2</td>
</tr>
<tr>
<td>3</td>
<td>Xylene (in condensate)</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>Risk Significant-Category 1</td>
<td>Adequate</td>
<td>C2</td>
</tr>
</tbody>
</table>
Based on the qualitative assessment results table above, it shows that the risk evaluation of benzene is included in the risk significant-category 2. Risk significant-Category 2 means that this hazard cannot be tolerated and must be eliminated, but if this is not possible, several efforts can be made such as the substitution of the hazardous chemical with a less hazardous chemical, total enclosure of process and handling system, or isolation of the work to control the emission of chemicals hazardous to health. Besides, the risk evaluation of toluene and xylene is included in the significant risk category-1. This means the risk has to be controlled to below permissible exposure limits or to as long as reasonably practicable (ALARP) where no limits are specified.

According to table 4, it shows that benzene, toluene, and xylene in condensate have a C2 conclusion, this means the risk is significant but adequately controlled. This conclusion applies to conditions where adverse health effects could increase in the future, due to control measure failure or deterioration; plant, equipment, or system failure; human error, from lack of awareness, monitoring failure or inadequate training; changes in methods or rate of work; and a significant increase in the number of chemicals hazardous to health used.

**Conclusion**

According to DOSH, 2000 the conclusion risk of Benzene, Toluene, and Xylene (in the condensate) is C2 which means the risk is significant but already adequately controlled could increase in the future. Therefore, several steps can be taken to control the risk of health hazards from benzene, toluene, and xylene in the condensate, for example: determine precaution to maintain controls and minimize chances of higher exposure occurring; determine additional measures for regaining control if a high-risk event occurs despite precaution; identify measures, procedures, and equipment to prevent or control any accidental emission of chemical hazardous to health, determine if monitoring or health surveillance is required to check on the effectiveness of controls; and review assessment every five years or when a change in circumstances.

Control measures are a way to reduce exposure to chemicals in labor. Prevention efforts that are carried out include eliminating chemicals that are hazardous to health, substituting hazardous chemicals with less hazardous chemicals, using controlled engineering equipment, isolation of hazardous chemicals, conducts health surveillance of workers, rotates employees if needed, and last uses personal protective equipment. Every control effort is carried out following the control hierarchy and according to the priority of chemical hazards that can be caused.

**Acknowledgements:** Thank you to all parties that are involved in the research, especially to the Laboratory of Natural Gas Company which has allowed the data collection.

**Ethical Clearance:** The study was approved by ethical clearance certificate Number 301 / HRECC. FODM / VI / 2020 from the Faculty of Dental Medicine Health Research Ethical Clearance Commission Universitas Airlangga.

**Conflict of Interest:** There is no potential conflict of interest

**Source of Funding:** The source of this research costs from self

**Reference**


A questionnaire-based Study of Knowledge and Attitude in Healthcare Professionals about Child Sexual Abuse related to POCSO (Protection of Children from Sexual Offences) Act: A Cross-sectional Study

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1Post Graduate Student (MD Forensic Medicine), 2Professor and Head (Department of Forensic Medicine), 3,4MBBS Student (third year), NIMS Medical College, Jaipur, Rajasthan

Abstract

Introduction: The Government of India has passed special law, The Protection of Children from Sexual Offences (POCSO) Act, 2012 which provides strong shield for protection of children from heinous crimes of sexual assault, sexual harassment and pornography, thereby safeguarding the interest of children at every stage of judicial proceeding.

Methodology: A questionnaire based study was carried among 100 healthcare professionals working at the tertiary care Hospital, randomly selected. The questionnaire had 15 Multiple Choice Questions about Child Sexual Abuse related to POCSO Act. Collected data was analysed statistically.

Objective: The study is aimed to assess knowledge and attitude about Child Sexual Abuse related to POCSO Act among healthcare professionals.

Result: Greater percentage of healthcare professionals have knowledge about major aspects of POCSO Act, as related to its full form (75%), enactment (62%), definition of child (62%), different types of sexual offence (86%), its mandatory reporting (81%), and trial in child friendly special courts (64%). But in few areas, knowledge related is scarce as only 40% participants knew punishment scales of POCSO Act and 46% knew failure to report is punishable offence. 84% participants suggest multidisciplinary approach is the need of hour to tackle cases of child sexual abuse.

Conclusion: To curb this menace educating health care professionals along with children, spreading knowledge and awareness at community level with strict law enforcement will play a key role.

Keywords: POCSO Act; Child Sexual Abuse; Knowledge; Attitude; Questionnaire; Healthcare Professional.

Introduction

The World Health Organisation (WHO) defines Child Sexual Abuse (CSA) as the involvement of a child or an adolescent in sexual activity that he or she does not fully comprehend and is unable to give informed consent to, or for which the child or adolescent is not developmentally prepared and cannot give consent, or that violates the laws or social taboos of society1. The CSA is one of the most alarming concern globally, as same holds true for India. India has one of the largest proportions of population in younger age groups globally. As per Indian Census 29.5% of the population of the country has been in the age group 0-14 years and 41% of the population account for less than 18 years of age2. Therefore nation bears huge responsibility to safeguard them from every kind of child abuse.

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E-mail: gnowsheen@gmail.com.
India’s first study on child abuse was reported in 2007, it reported 53.22% children having faced one or more forms of sexual abuse and most of children did not report the matter to anyone. Crime against children in India is following steady upward trend with a significant increase of more than 500% over a period of the past one decade (1,06,958 cases in 2016 over 18,967 cases in 2006), where Uttar Pradesh tops the list with 15% of recorded crimes against children, Maharashtra and Madhya Pradesh closely follow with 14% and 13% respectively.

The children who are promising future of a country are suffering inequity mainly in developing countries among which sexual abuse is the most heinous crime. In 2012, Indian government enforced a revolutionary act in Protection of Children from Sexual Offences (POCSO) Act, which has demarcated sexual abuse in child from that of adult. In India prior to enactment of POCSO Act there was no child specific act related to CSA followed nationwide. The Goa Children’s Act, 2003, was the only child abuse confined law with its state specific enactment in Goa. All the crimes related to child sexual abuse were punished under various Indian Penal Code (IPC) legislations (IPC 375- rape, IPC 354- outraging modesty of woman, IPC 377- unnatural sexual offences) were put in force for dealing with child sexual abuse. The POCSO Act brought all forms of sexual offences related to children under one umbrella of legislation right from reporting to judgement. The principle POCSO Act, 2012 is a comprehensive law which provides the protection of children from the offences of sexual assault, sexual harassment and pornography, while safeguarding the interest of the child at every stage of the legal proceedings by incorporating child-friendly system for reporting, recording of evidence, investigation and speedy trial of offences through designated Special Courts. It defines a child, as any individual below the age of eighteen years with no gender bias which is in accord with UN Convention on Rights of Children, the most validated children rights treaty worldwide. The POCSO Act identifies six types of sexual offences in children:

1. Sexual Assault: When a person with sexual intent touches the vagina, penis, anus or breast of the child or makes the child touch the same for that person or someone else. The punishment is minimum imprisonment of 3 years which can go up to 5 years and fine.

2. Aggravated Sexual Assault: When the sexual assault is carried by a person who in position of trust like relative, police officer, a member of armed force, public servant, management or staff of hospital or educational or any other religious institution. The punishment is minimum imprisonment of 5 years can go up to 7 years and fine.

3. Penetrative Sexual Assault: Whoever causes insertion of penis/ object/ another body part in child’s vagina/ urethra/ anus/ mouth, or asking the child to do so with him or someone else. The punishment is minimum imprisonment of 7 years which can go up to life-time imprisonment and fine.

4. Aggravated Penetrative Sexual Assault: When the penetrative sexual assault is carried by a person who in position of trust like relative, police officer, a member of armed force, public servant, management or staff of hospital or educational or any other religious institution, it constitutes aggravated penetrative sexual assault. It also covers any grievous hurt caused to child, penetrative sexual assault on already pregnant child, repeated assault and penetrative assault on child below the age of 12 years. The punishment is minimum imprisonment of 10 years which can go up to life-time imprisonment and fine.

5. Sexual Harassment: Whoever passes sexuality related remark, sexual gesture/noise, repeatedly following, flashing, or makes the child exhibit any part of his body so as it is seen by such person or any other person. The punishment is imprisonment up to period of 3 years and fine.

6. Using Child for Pornographic Purposes: Whoever uses child in any form of media for the purpose of sexual gratification. The punishment is imprisonment up to 5 years and fine.

According to National Crime Record Bureau, crime against children is increasing in India each successive year, in major metropolitan cities total of 18247 cases of crime against children were seen in 2015, which increased to 19081 cases in 2016, further increased to 19544 in year 2017. Moreover, in 2018 records of NCRB India, crimes under POCSO Act were among leading crime heads of Crime against Children and contributed 34% to it. The rate of crime has increased per lakh children
population from 28.9 in 2017 to 31.8 in year 2018\textsuperscript{10}, such a rising surge each year where every single rise in number means silent massacre of innocence. It led Government of India take great initiative to amend POCSO Act by introduction of capital punishment for aggravated sexual offences and adding various new clauses to the principle act making it more stringent with respect to punishment standards\textsuperscript{11}. To control this endless crime, with the efforts of Government in formulation and implementation of new legal provisions, public knowledge and attitude are equally significant as procedural tools in preventing and eliminating such offences.

**Material and Methods**

Setting: This study was conducted at National Institute of Medical Sciences and Research (NIMS) Hospital, a tertiary care Hospital in Jaipur.

Study design: The study design is cross sectional questionnaire-based study. Participants of study consisted of 100 healthcare professionals (doctors) working at NIMS Hospital in Jaipur. The survey conducted is about knowledge and attitude about CSA related to POCSO Act in health care professionals. These professionals were randomly selected from all those working at NIMS hospital, with only criteria of minimum MBBS (Bachelor of Medicine and Bachelor of Surgery) qualification. The questionnaire had multiple choice questions (MCQs) related to POCSO Act, these were distributed among participants in their respective department with the help of volunteers from undergraduate students of third-year MBBS. The questionnaire comprised of basic demographic profile along with 15 multiple choice questions, among which first 12 questions were related to knowledge, two related to attitude and one related to source of information. Pretesting of questionnaire was done on 20 randomly selected healthcare professionals of the hospital. The questionnaire was finalised after modifying unsuitable questions based on the pre-test result.

Data collection: One hundred and twenty questionnaires were distributed among healthcare professionals from which 100 respondents were included in the study, who filled all criteria of questionnaire properly remaining were discarded. One day was given for data collection with no specific time followed.

**Results**

Response rate was 83% as out of 120 distributed questionnaires among which 100 participants responded properly fulfilling all criteria and same were included in study population. Demographic details of health care professionals are summarized in Table No. 1.

<table>
<thead>
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<th>Table No.1: Demographic details of healthcare professionals (n=100)</th>
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</tr>
<tr>
<td>Female</td>
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<td><strong>Age distribution (in years)</strong></td>
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<tr>
<td>51-60</td>
</tr>
<tr>
<td>61-70</td>
</tr>
<tr>
<td><strong>Professional qualification</strong></td>
</tr>
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<td>MBBS (Bachelor of Medicine and Bachelor of Surgery)</td>
</tr>
<tr>
<td>PGT (Post Graduate Trainee)</td>
</tr>
<tr>
<td>MD/MS (Doctor of Medicine / Master of Surgery)</td>
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</table>
Table No.1: Demographic details of healthcare professionals (n=100)

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<th></th>
<th>Married</th>
<th>Single</th>
<th>Residence</th>
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<tbody>
<tr>
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<td>38</td>
<td>62</td>
</tr>
<tr>
<td>Residence</td>
<td>85</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

As per knowledge based questions asked, 75% healthcare professional knew the abbreviation POCSO stands for. Both the year in which POCSO was enacted and the age related definition of child under POCSO Act was known to 62% participants. 86% of healthcare workers knew offences that come under POCSO Act. 81% participants knew that reporting child sexual abuse is mandatory but only 46% had knowledge that failure to report is punishable offence. Only 40% of healthcare professional were aware that punishment is graded as per magnitude of offence under POCSO Act. Trial of cases under POCSO Act are held at child friendly Special courts was correctly marked by 64% people. 95% participants were aware that POCSO Act is gender neutral. Child helpline number of India was known to 64% of healthcare professionals. 72% professional knew Child sexual abuse is punished under POCSO Act. Only 56% healthcare workers were aware that POCSO Act was amended in 2019. Figure No.1 gives graphical representation of knowledge-based response in healthcare professional.

The present study regarding attitude of healthcare professionals about CSA revealed, 4% healthcare professionals opine Government Organizations will play a vital role in supporting sexually abused child while as 9% say Non-Government Organizations (NGO) will, whereas 21% healthcare workers say child welfare committee should play the key part, but majority of healthcare professional 66% suggest that community as a whole including fore mentioned all Organizations together should play vital role to support sexually abused child. In view of 5% participants it is educating the children from primary level is need of hour to reduce CSA, 8% respondents say strict law enforcement is most important to follow, 3% suggest spreading of awareness.
at community level is more important, whereas majority of healthcare workers (84%) agree that collective effort of all of the above measures is required. Data is represented in graphically in Figure No.2.

![Figure No. 2: Attitude-based response given by Healthcare Professionals](image)

Lastly, about source of knowledge about POCSO Act, 41% of respondents said it was a teacher at an educational institution who provided them knowledge about POCSO Act, 33% got it from electronic media (internet, television, radio, etc.), 19% from print media (newspapers, journals, books, etc.) Whereas family and friend discussion in 7% respondents was the source of information related. Graphically shown in Figure No.3.

![Figure No. 3: Source of knowledge related to POCSO Act among Healthcare Professionals](image)

### Discussion

Child sexual abuse is menace worldwide, making it issue of global concern with short-term and life-long consequences on the physical as well as mental health of a child. An epidemiological overview by Mannat M Singh et al (2014) reported the prevalence of CSA was found to be high in India as well as throughout the world. CSA is an extensive problem and even the lowest prevalence includes a huge number of victims. The POCSO Act practices no discrimination in the provision of care towards abused child, irrespective of sex, race, ethnicity, religion, sexual orientation, and socioeconomic status.
which is in accordance with WHO guidelines.7,13 The World Health Organization (WHO) has been working for years to challenge gender stereotypes and combat gender inequality, identify and eliminate gender-related barriers to healthcare, and implement gender-responsive care. Knowledge about gender neutrality of victim in POCSO Act was reported by 95% of respondents in our present study which shows promising number for good practice when dealing with CSA cases.

Mandatory reporting under POCSO Act will break the silence on child sexual abuse which is still a taboo, greater number of concealed cases will pop up and verdict of justice will prevail. In India a large number of cases on child sexual abuse go unreported due to socio-cultural and ethical stigma related to it. Delanthabettu et al in 2017 conducted a questionnaire based study on Child Sexual Abuse in school going children, which showed among 1336 students, 165 reported sexual abuse, among which 51.7% victims remained silent or ignored it while just 17.2% children informed their parents. This silence encourages the perpetrators to become more fearless and continue the more severe forms of sexual abuse. But under POCSO Act, failure to report any case of child sexual abuse is punishable offence, one may be punished with imprisonment up to period of six months with or without fine.7 Same ideology is put forth by Paradise J S in his article related to CSA that the physicians must report all cases of suspected sexual abuse to United States child protective services agencies as failure to do so can incur legal penalties.15 This shows not only in India but in other countries too reporting of CSA is mandatory. Another study on CSA by Arya and Chaturvedi (2017) reported that lack of proper training to handle the victim of CSA may add to the problems of doctors.16 Therefore knowledge about POCSO Act in doctors is not only important from victims prospective but also for safeguarding themselves from the legal penance.

Government of India has shown many great effort in safeguarding rights of children, one among which is Child helpline, 1098. This child-line is developed and managed by CIF (Child India Foundation), it is a 24x7, emergency, free phone outreach service linking children in need of care and protection, to relevant organisations run by government departments and civil society organisations for long-term care, protection and rehabilitation.17 This helpline has been life saviour for many victims as its easy accessible one point contact between distressed person and responsible authorities. Along with parents and guardians, people who are related to children and their rights need to be aware about it, among our study good percentage (62%) of participants had knowledge about this child-line number.

Healthcare professionals are first-line support to sexually abused children. As per WHO guidelines regarding first- line primary psychological support to all children and adolescents who disclose sexual abuse, a healthcare provider should be able to provide minimum level of psychological support with validation of their experience.18 In accord with same Indian legislation has set forth the guidelines under POCSO Act for medical professionals, who are to deal with sexually abused children.19 Medical practitioner who provides medical assistance to victim is liable to show proficiency in: (1) history taking in child friendly manner; (2) collection of evidence after a thorough medical examination; (3) treatment of physical and genital injuries if present; (4) evaluation for mental health status and referral for psychiatric counselling sessions if required. Sometimes age assessment of victim is also mandated by court. A study by Moirangthem S et al (2015) reported issues and concerns related to child sexual abuse, which stated training all the stakeholders is one of the important variables in providing comprehensive care and justice under POCSO Act.20 In the special article related POCSO Act by Seth and Srivastave (2017) supported same fact stating Paediatricians and health care professionals need to acquire necessary expertise for clinical evaluation of CSA, as they are usually first point of contact with sexually abused child.21 Thus initiatives for training of practising health care professionals along with medical students should be considered important for better structured approach of abused victims.

Punishment section of every crime holds great importance, as it keeps crime in check. Same is true for CSA, for which in India POCSO Act defines punishment scale. Our present study result showed poor awareness in health care professionals about punishment section and amendment (2019) of POCSO Act. Similar results were observed by Singh Jaswinder et al (2019) who aimed to ascertain level of knowledge and perceptions in MBBS course students at Bareiley, which reported
limited knowledge in participants related to legal aspects of CSA, reporting and preventive measures of such crimes. Another research study report by Kailash Satyarthi Children’s Foundation (KSCF) on Awareness and Perceptions about Child Sexual Abuse among 987 young adults of India disclosed three out of every four young adults do not possess a basic understanding about CSA, very few were aware that even teasing is a crime. Among surveyed participants 90% were aware that the CSA is a punishable offence but only 72% had awareness of the POCSO Act, which shows similarity with our study results where 75% of healthcare professionals had awareness about POCSO Act. Study by Minakshi Bhosale et al (2018) in medical students about CSA showed variance with our study results as it reported only 20.4 % participants were aware about the POCSO Act. Therefore, such dispersed result data suggest that there is still an immense need to aware general population along with medical professionals about CSA and related legislation (POCSO Act), as awareness about sexual abuse can go a long way in prevention of CSA. The Gupta et al (2013) reported POCSO casts a duty on the central and state governments to spread awareness through media including the television, radio and the print media at regular intervals to make the general public, children aware of the provisions of this act. The study by KSCF reported electronic and print media, were main source of information to respondents about CSA whereas our study reported 41% respondents had a teacher who was their primary source of information about POCSO Act closely followed by electronic media in 31% respondents.

Conclusion

Basic knowledge regarding POCSO Act is present in majority of healthcare professionals but knowledge related to deeper aspects is scarce which includes punishments related to POCSO Act and amendments it has gone through. This knowledge gap could be filled by introducing POCSO Act in basic MBBS curriculum and to refresh same in practising medical professionals by conducting regular CME (Continued Medical Education), seminars, symposiums and workshops related to CSA mainly focusing on POCSO Act. Nonetheless educating children from primary level about good and bad touch, spreading knowledge and awareness at community level is equally important to curb CSA. Multidisciplinary approach involving primary healthcare workers, police officers, legal agencies, lawyers, child welfare committee workers, paediatricians, gynaecologists, forensic experts, psychiatric specialists, NGOs, are essential to handle the cases of CSA for its better structural approach and improved outcome. Moreover, for rehabilitation of sexually abused children supportive environment at school and home need to be nurtured.

Acknowledgement: We would be obliged and thankful to all the medical-practitioners who cooperated with us and became part of our research study.

Conflict of Interest: All the authors declare that they have no conflict of interest.

Informed Consent: Informed consent was obtained from all the study participants.

Ethical Approval: The study was carried out with ethical standards of institution.

Funding Source: No relevant financial interests to disclose.

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6. Indian code, The Indian Penal Code 1860. Available from: https://www.indiacode.nic.in


Level of Knowledge and Family Support toward Medication Adherence among Patient with Diabetes Mellitus in Malang, Indonesia

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1Lecturer, 2Nursing student, Department of Community Health Nursing, Faculty of Health Science, University of Muhammadiyah Malang, Jl. Bendungan Sutami 188A Malang, Indonesia

Abstract

Patient compliance with long-term therapy for chronic diseases in developing countries is still low. Efforts to control blood sugar levels remain normal with medication adherence depending on family support and patient knowledge of the disease. This study aimed to determine the relationship between the level of knowledge and family support with medication adherence in patients with diabetes mellitus in Malang, Indonesia. The study design was cross-sectional. The sample of this study was 48 people with diabetes mellitus recorded in Ciptomulyo Health Center. This study uses a simple random sampling technique. The questionnaire used consisted of demographic data, knowledge, family support, and medication adherence. The results of the study were that there is no relationship between the variable level of knowledge and medication adherence in patients with diabetes mellitus with a significance value of p = 0.561. There was a relationship between family support and medication adherence in patients with diabetes mellitus with a significance value of p = 0.000 and a Correlation Coefficient value of 0.616 which indicates a significant relationship with a strong degree of relationship strength. There is a relationship between family support and medication adherence in patients with diabetes mellitus at the Ciptomulyo Health Center. The better the family support, the more obedient the patient is undergoing treatment.

Keywords: Knowledge, Family Support, Medication adherence, Diabetes Mellitus

Introduction

The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%). Indonesia became Top 10 countries or territories for number of people with diabetes (20–79 years) in 2019, 2030 and 2045. In addition to the world and Indonesia level, the increase in the incidence of Diabetes Mellitus in the data based on the diagnosis of doctors, the prevalence of Diabetes Mellitus in the population of all ages by the province in 2018 especially in the province of East Java was recorded as much (2%), the average age of 55-64 years sufferers (6.3%), male (1.2%) while the female (1.8%). In urban areas more (1.9%) and rural areas only (1.0%). The prevalence of Diabetes Mellitus according to the 2015 Consensus consensus in the population aged ≥ 15 years was recorded at 10.9%. According to WHO, it is predicted that there will be an increase in Diabetes sufferers in Indonesia from 8.4 million in 2000 to 21.3 million in 2030.

WHO reports that the average patient adherence to long-term therapy for chronic diseases in developing countries is still low, whereas in developed countries it reaches 50%. Several reasons can affect diabetics who do not routinely take OAD / insulin injections, among others: feeling healthy, not routinely going to a health care facility, taking traditional medicine, often forgetting, not being able to take side effects, not being able to buy...
drugs regularly, and the unavailability of drugs in health care facilities. Successful management of Diabetes Mellitus can be seen from adherence to the glucose diet and also depends on the patient himself. Things that can affect the attitude in going on a diet or treatment are adequate levels of knowledge from sufferers about their illness, controlling blood sugar levels, and preventing complications to live healthier and more quality.

Compliance with medication in patients with diabetes mellitus against treatment programs that have been recommended by doctors or health workers can worsen the condition of the disease. Efforts to control blood sugar levels remain normal also depend on motivation, family support, and patient knowledge of the disease. Someone who is making a choice is also related to the knowledge that is related to the behavior that will be taken. The knowledge in question is the patient’s knowledge about the causes, signs, how to treat, and where to seek treatment. Meanwhile, family support can increase a patient’s positive emotional response to the management of his illness due to the help and motivation. Patient compliance with taking drugs plays a very important role in the success of treatment to maintain blood glucose levels and blood pressure in the normal range.

The purpose of this study was to determine the relationship between the level of knowledge and family support with medication adherence in patients with diabetes mellitus. The results of this study can be used as a reference for community nurses in providing education for people with diabetes mellitus about the importance of increasing knowledge about their illnesses and the importance of family support for adherence to taking medication for people with diabetes mellitus. As a result, blood sugar levels remain stable and normal.

**Method**

**Study design and Sampling**

The research method used is a correlational research design with a cross-sectional approach. The sampling technique used in this study was carried out with the Simple Random Sampling technique. Samples obtained as many as 48 respondents from 54 populations in RW 2. The number of samples was obtained from the results of calculations using the Slovin formula.

**Level of knowledge questionnaires**

This questionnaire uses The Diabetes Knowledge Questionnaire (DKQ-24) related to the client’s knowledge of the cause of the disease, symptoms or signs of the disease, how to treat or where to seek treatment by respondents who were adopted from the Menino (2017) that was modified by the researcher. This questionnaire has 24 statement items. In this questionnaire using a scoring of 3 categories that are worth <56% (Less), 56% -75% (Fair), 76% -100% (Good) with a score of 1 correct answer and 0 on the wrong answer.

**Family support questionnaires**

Family support for Diabetes Mellitus patients was measured using a social response questionnaire adopted from Kurniawan (2016) and modified by researcher. This questionnaire has 4 criteria for answers starting from options always to never using multiple-choice types. This family support questionnaire includes 3 family support domains with 12 question items. The scope of the family support domain in this questionnaire includes the domain of informational support, instrumental support, and emotional support and self-esteem. The scoring in this questionnaire uses four scales worth 1-4. Value 1 (never), 2 (rarely), 3 (often) and 4 (always). The highest total score is 48 and the lowest is 12.

**Medication adherence questionnaires**

Medication adherence for patients with diabetes mellitus measured using MMAS-8 (Morisky Medication Adherence Scale) is used to assess compliance with taking drugs that have been translated and validated in the Indonesian version by Ardanti (2016) about research into drug adherence in DM patients consisting of four aspects i.e. forgetting / not taking medicine as much as 4 questions with item number 1,2,4,5; stop taking medicine for 2 questions for items number 3 and 6; treatment interferes with 1 question on item number 7 and it is difficult to remember taking medicine on item number 8. This questionnaire contains 8 questions, each question has a choice of the answer “yes” or “no” and one question with 5 Likert scales (never / rarely, several times, sometimes, often and always). Response categories consist of “yes” or “no” for question items number 1-8. In question items, number 1-4 and 6-8 the value is 1 if the answer is “no” and 0 if the answer is
“yes”, while question number 5 is valued 1 if “yes” and 0 if “no”. The interpretation of this questionnaire is stated to be compliant (value = 8), less compliant (value = 6-7) and non-compliant (value = <6) 11.

Data Analysis

Research data were analyzed using descriptive and analytical analysis with the Spearman Rank correlation test. Data analysis using Statistical Package for Social Sciences (SPSS) software version 23.0. A p-value <0.05 was considered statistically significant.

Results

Demographic characteristics

Table 1: Demographic data of the respondents (n=47)

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<td></td>
<td>Employment</td>
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<td>31,2</td>
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Level of knowledge

Table 2 showed that the majority of respondents had a lack of knowledge, which is 69% (n = 33).
Table 2: Level of Diabetes knowledge (n=47)

<table>
<thead>
<tr>
<th>Level of knowledge</th>
<th>Frequency</th>
<th>Percentage (%)</th>
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<td>Good</td>
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<td>Moderate</td>
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<td>Poor</td>
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<td>69</td>
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</tbody>
</table>

Family support

Table 3: Family support of the respondent (n=47)

<table>
<thead>
<tr>
<th>Family support</th>
<th>Frequency</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Good</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Poor</td>
<td>34</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 3 explains that the frequency of family support variables in patients with diabetes mellitus is in the poor category by 34 people (71%) and the good category by 1 person (2%).

Table 4: Each dimension of family support

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Category</th>
<th>Low (%)</th>
<th>Moderate (%)</th>
<th>High (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informational</td>
<td>Low</td>
<td>23</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>Instrumental</td>
<td>Low</td>
<td>0</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Emotional</td>
<td>Low</td>
<td>23</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 4 explained that the dimension of informational family support has a high percentage compared to other family support, which is as much as (46%).

Medication adherence

Table 5: Medication adherence of the respondent’s (n=47)

<table>
<thead>
<tr>
<th>Medication adherence</th>
<th>F</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>52</td>
</tr>
<tr>
<td>Poor</td>
<td>15</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 5 explained that the frequency of medication adherence variables in patients with diabetes mellitus is in the category of poor adherence as many as 25 people (52%) and the category of non-adherent as many as 15 people (31%).
Crosstabulation of the level of knowledge, family support, and medication adherence

Table 6 shows the results between the level of knowledge and family support with medication adherence in patients with diabetes mellitus, it is known that the highest score on the variable level of knowledge is in the category of lack of knowledge and adherence to taking medication that is less adherent as many as 19 respondents from 48 respondents. Meanwhile, the family support variable is lacking in the category, and adherence to taking medication that is not compliant is 20 respondents out of 48 respondents.

Table 6: Crosstabulation of the level of knowledge, family support, and medication adherence

<table>
<thead>
<tr>
<th>Level of Knowledge</th>
<th>Medication adherence</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Moderate</td>
</tr>
<tr>
<td>Poor</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Good</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family support</th>
<th>Medication adherence</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Moderate</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Good</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>25</td>
</tr>
</tbody>
</table>

Correlation between Level of knowledge and medication adherence

Based on table 7, it is known that the Spearman rho correlation test results obtained p-value = 0.05 smaller than alpha (0.05), then meaning that there is no relationship between the level of knowledge and adherence to taking medication with patients with diabetes mellitus.

Table 7: Correlation between Level of knowledge and medication adherence

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Level of knowledge</th>
<th>Medication adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman’s rho</td>
<td>Level of knowledge</td>
<td>Correlation Coefficient Sig. (2-tailed) N</td>
</tr>
<tr>
<td></td>
<td>Medication adherence</td>
<td>Correlation Coefficient Sig. (2-tailed) N</td>
</tr>
</tbody>
</table>
Correlation between family support and medication adherence

Based on table 8, it is known that the Spearman's rho correlation test results obtained p-value = 0.005 smaller than alpha (0.05), then there is a relationship between family support and medication adherence with patients with diabetes mellitus. The correlation coefficient (0.616) shows the level of relationship between the variables of family support with medication adherence in patients with diabetes mellitus included in the category of strong correlations.

<table>
<thead>
<tr>
<th>Table 8: Correlation between family support and medication adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation</strong></td>
</tr>
<tr>
<td><strong>Spearman’s rho</strong></td>
</tr>
<tr>
<td><strong>Family support</strong> Correlation Coefficient Sig. (2-tailed) N</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Medication adherence</strong> Correlation Coefficient Sig. (2-tailed) N</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The results showed that the majority of respondents had a lack of knowledge. The level of knowledge can be influenced by several things, such as experience, education level, beliefs, and facilities. The majority of respondents in this study fall into the category of adult age, adulthood is the age at which individuals re-evaluate life experiences and redefine themselves in their roles and values. This is confirmed by research conducted by Sulistin (2015) using adult respondents with a high level of knowledge and there is a significant relationship between the level of knowledge and people’s attitudes about schistosomiasis. Research respondents who are in RW 2 Ciptomulyo Public Health Center in Malang, it can be concluded that diabetes mellitus patients have a lack of knowledge, this can be influenced by age and education factors, where the majority of patients including 28 elementary school graduates. In line with a previous study reported that people with diabetes mellitus had poor level in diabetes knowledge (31.5%). Based on the results of the study, it was found that the family support of patients with diabetes mellitus at the Ciptomulyo Health Center is classified in the poor category. This is likely due to instrumental and emotional family support being classified as low. This study in line with Iloh (2018) that reported 22.5% did not have family support among diabetic patient.

It was found that adherence to taking medication in patients with diabetes mellitus was classified as less compliant as many as 25 people (52%). According to Alqarni (2019) reported that 21.4% had low medication adherence among diabetic patients in the Bisha governorate of Saudi Arabia. Factors recorded in this study, which can be called influential on adherence to taking medication for patients with diabetes mellitus are knowledge, which can be seen from the data filling questionnaire level of knowledge that the majority of patients included in the category of fewer than 33 people (69%).

The results of this study can be concluded that the relationship between the level of knowledge with medication adherence in patients with diabetes mellitus in Ciptomulyo Health Center is uncorrelated because the variable level of knowledge has a significance value > 0.005 and with a very weak correlation strength. In this
study, the variable level of knowledge with medication adherence does not have a significant relationship because Factors recorded in this study are internal factors, it was found that the majority of patients with diabetes mellitus in Puskesmas Ciptomulyo graduated from elementary school as many as 28 people (58.3%), not working as many as 33 people (68.8%), aged 18-65 as many as 34 (71%). These factors affect the level of knowledge of patients regarding adherence to taking the medication they live. So it can be concluded that in this study Ho was accepted that there was no relationship between the level of knowledge with medication adherence in patients with diabetes mellitus in Ciptomulyo Health Center.

Based on the results of the study, it is known that the highest score on the variable family support with the category of lack and adherence to taking the category of medication less adherent as many as 20 respondents from 48 respondents. Developmental stage in patients with diabetes mellitus in Puskesmas Ciptomulyo RW 2, including at the stage of family life with old age, at which stage the patient adjusts to losing a partner and maintains intergenerational ties, so that the majority these patients only live alone and are often left traveling by family and children, both for work, family matters, etc. Meanwhile, in terms of education and the level of knowledge of the majority of diabetes mellitus patients in Ciptomulyo Public Health Center there are 28 elementary school graduates (58, 3%) and with a level of knowledge of the category of fewer than 33 people (69%). Results from the Spearman Rank correlation test can be concluded that family support with medication adherence in patients with diabetes mellitus correlates with a significance value <0.005 and with sufficient correlation strength. The results of tests that have been carried out can be concluded that the better the support provided by the family will increase the compliance to take medication for patients. In line with a previous study that perceived family members engage in more unsupportive behavior is associated with less adherence to one's diabetes treatment regimen, and being less adherent is associated with poorer glycemic control. 17

Conclusions

There is no relationship between the level of knowledge and adherence to taking medication and the relationship between family support with adherence to taking medicine that is with the strength of a strong relationship. In mean that family support should be improved to provide medication adherence among diabetes patient. Family support have a main role improving medication adherence among patient with diabetes mellitus. As a result, nurse should work together with the family member to prevent the diabetes complication through medication adherence,

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: This research has been registered in Malang City Health Service No. 072/849/35.73.302/2019

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Influence of Exercise Classics on the Development the Volume of Attention of Schoolchildren Aged 8-9

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Abstract

The aim of the study: To determine the influence of exercise Classics on the development of the volume of attention of children 9-10 years old in physical education lessons.

Methods and Material: The study was conducted over a period of 9 months, in which 40 9-10-year-olds took part. Physical education classes were held 2 times a week for 40 minutes each lesson. The level of development of coordination abilities was assessed on the «Shuttle run» test, and the indicators of attention volume on the «Schulte Table» test. The programs bio-stat 2009, Microsoft excel 2016 and t-student were used for mathematical and statistical processing of results.

Results: Before the beginning of the pedagogical experiment, the indicators of school children between the groups did not have significant differences (P>0.05). After the end of the study, the indicators in both groups improved. In CG, in the «Shuttle run» test, the indicators improved from 10.2±0.6 to 9.9±0.5 (P>0.05), and in the volume of attention test, the indicators improved by 6.1% (P>0.05). In EG, in the «Shuttle run 3x10 m» test, the indicators improved from 9.9±0.5 to 8.5±0.4 (P<0.05), and in the «Schulte Table» test, the indicators improved by 32.1%. These results indicate the effectiveness of using exercise Classics in physical education lessons in working with younger schoolchildren.

Conclusion: if schoolchildren will perform exercise Classics in physical education classes at school, they will improve not only coordination abilities, but also the volume of attention.

Key Words: volume of attention, coordination abilities, schoolchildren, physical education, Classics.

Introduction

A healthy lifestyle includes several components, including motor activity. It should be noted that insufficient motor activity, even in childhood, can lead to serious negative consequences for health. Unfortunately, there are more and more first-graders who have abnormalities in their health, usually due to lack of movement. Physical education at school is an integral part of the modern education system. The main form of physical exercises in school is a physical culture lesson. At the lesson, schoolchildren receive the necessary minimum of knowledge, skills, and skills provided for in the school curriculum, and increase their level of physical development. The main goal of the lesson is that children, starting from their earliest childhood, grow up healthy, strong, harmoniously developed, so that they learn well. Physical culture plays a major role in improving a person’s health1,2.

Today, there is a physical education program for schoolchildren from the first to the eleventh grade. In each age group, a detailed description of the load on schoolchildren and the exercises that they must master is given3.

Despite the versatility of modern physical education programs, they have shortcomings, for example, to implement some tasks, you need a large gym, which is not in every school, there is not enough space for physical education, which reduces the motor density of classes. How to increase physical activity of children in such conditions? Despite the fact that some authors suggest replacing the standard program with modern
methods, we believe that it will only be enough to slightly supplement the existing program. It is proved that the exercise Classics allow you to solve this problem.

Exercise Classics allow you to implement an individual and differentiated approach to schoolchildren, which is important for each of them. It allows you to open and increase the reserve capabilities of physical abilities.

We should also mention the development of coordination abilities for children of primary school age. Coordination abilities are the ability of a person to quickly master new movements and reconstruct motor activity in accordance with the requirements of a changed situation. The level of development of coordination abilities determines the effectiveness of human movement, its rationality of actions, speed and other aspects of movement. Coordination abilities are also necessary for mastering movement techniques in any sport. The higher the level of development of coordination abilities, the more effective the athlete will act.

At the age of 7-11 years, it is necessary to lay the Foundation for the growth of the development of the body and abilities involved in school, since this age is favorable for the development of most physical abilities.

Physical education and sports have a positive impact on the cognitive processes of schoolchildren. However, despite the established relationship, it is important to study the influence of Classical exercises on the volume of attention of schoolchildren.

Aim of the study – to determine the influence of exercise Classics on the development of the volume of attention of children 9-10 years old in physical education lessons.

The hypothesis of the study. It is assumed that if you use the Classic exercise in physical education classes, the indicators of the volume of attention of schoolchildren will improve.

**Material and Methods**

**Participants:**

Boys and girls 9-10 who participated in the study were healthy and admitted to physical education classes. The schoolchildren studied in grade 3, at secondary school Number 60, in the city of Kirov (Russia).

All procedures conformed to the ethical standards of the Helsinki Declaration of 1964. Informed consent was obtained from all parents of schoolchildren included in the study.

**Procedure:**

The study continued for nine months (September-may). A total of 56 physical education classes were held. Classes were held 2 times a week. Each lesson lasted for 40 minutes.

Prior to the study, 2 study groups were formed:

1. the Control group (CG) consists of 20 children from class 3A. These schoolchildren were engaged in a standard physical education program at school.

2. Experimental group (EG) – children of the 3B class in the amount of 20 people. These schoolchildren were engaged in a standard program, but in addition to each lesson, they performed an exercise Classics. The exercise is presented in table 1.

**Table 1. Exercise «Classic’s»**

<table>
<thead>
<tr>
<th>5</th>
<th>1</th>
<th>6</th>
<th>8</th>
<th>6</th>
<th>1</th>
<th>1</th>
<th>7</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>2</td>
<td>8</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Square 1  Square 2  Square 3
**Exercise Classics:**

In the gym, there are three large squares on the floor. The side of one square is 180 cm. Inside each large square there are nine small squares, the side of the small square is 60 cm. Inside each small square are numbers from 1 to 9.

Task: the schoolchild must use jumps from square to square to get from number 1 to number 2, then to number 3, and so on, to number 9. After that, it should jump on the same squares in reverse order (from number 9 to number 1). You can move around the squares in any way (from one leg to the other, jump on one leg or on two). If the schoolchild makes a mistake, he returns to the previous square. During the lesson, each schoolchild must overcome three large squares. The numbers in the squares must be changed by the teacher before each lesson. You can perform the exercise in any part of the lesson.

Before and after the pedagogical experiment all schoolchildren took control tests:

1. «Shuttle run 3x10 m» (indicator of coordination abilities)\(^{14}\).

2. «Schulte Tables» (indicator of the volume of attention)\(^{15}\).

On an ordinary sheet of A4 paper, 25 cells are drawn, and the numbers 1-25 are randomly written in each cell (table 2).

### Table 2. Schulte Tables

<table>
<thead>
<tr>
<th></th>
<th>10</th>
<th>21</th>
<th>16</th>
<th>2</th>
<th>19</th>
<th></th>
<th>21</th>
<th>6</th>
<th>14</th>
<th>7</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>---</td>
<td>----</td>
<td>---</td>
<td>----</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>1</td>
<td>11</td>
<td>3</td>
<td></td>
<td>19</td>
<td>25</td>
<td>23</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>12</td>
<td>17</td>
<td>6</td>
<td>7</td>
<td></td>
<td>11</td>
<td>17</td>
<td>1</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>20</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td></td>
<td>20</td>
<td>2</td>
<td>12</td>
<td>18</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>13</td>
<td>9</td>
<td>5</td>
<td>18</td>
<td></td>
<td>13</td>
<td>22</td>
<td>3</td>
<td>9</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Option 1
Option 2

At the signal, children must cross out all the numbers in order, from 1 to 25. After the last digit, the test stops. The result is the time that the schoolchildren completed the task. One attempt. If an error is made, the test is repeated.

**Statistical Analysis**

In the course of the study, we used the program Microsoft excel 2016, and bio-stat 2009. The detection of arithmetic averages in the tests of both groups was carried out using Microsoft excel 2016. Bio-stat 2009 allowed using the parametric criterion t-student (confidence at P<0.05)\(^{16}\).

**Results**

Before the beginning of the pedagogical experiment, schoolchildren from class 3A and 3B passed two control tests Shuttle run 3x10 m and Schulte Tables. The difference between the indicators was not significant (P>0.05). However, after the pedagogical experiment, the indicators in both groups differed significantly (table 3).
Table 3. Indicators of coordination abilities and attention volume children 9-10 years old

<table>
<thead>
<tr>
<th>Test</th>
<th>CG</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Shuttle run 3x10 m (s)</td>
<td>10.2±0.6</td>
<td>9.9±0.5</td>
</tr>
<tr>
<td>Schulte Table (s)</td>
<td>37.7±4.7</td>
<td>35.4±5.1</td>
</tr>
</tbody>
</table>

Table 3 shows that the results of 9-10 year olds in both groups changed from the beginning to the end of the study. The performance of coordination abilities in the «Shuttle run 3x10 m» test in CG improved, but not significantly from 10.2±0.6 to 9.9±0.5 (P>0.05), in contrast to children from class 3B. In EG, the indicators of coordination abilities improved from 9.9±0.5 to 8.5±0.4 (P<0.05). The situation in the «Schulte Table» test is the same. The volume of attention of schoolchildren in class 3A improved, but only by 6.1% (P>0.05), and for schoolchildren in class 3B by 32.1% (P<0.05). Such results may indicate that the standard physical education program at school is not very effective, at the same time, the results in EG show the effectiveness of implementing exercise Classics in the educational process of younger schoolchildren, which significantly improved the indicators of coordination abilities and the volume of attention.

Discussion

Children’s health is an important issue in society today. This problem can be solved, first of all, by physical education and physical exercises. In General, physical education is aimed at a comprehensive harmonious development of a person and his preparation for work in life\cite{1,2}. In the Russian Federation, physical education classes at school are mandatory. And the physical education program at school covers a very large set of skills and abilities of physical abilities of children, which need to be developed through physical exercises\cite{3}. However, some authors believe that the standard physical education programs at school are very outdated and they need to be completely replaced with modern methodics and new sets of exercises\cite{4}. This approach seems to us not quite correct, the correct option would be to Supplement existing programs. One of the most effective additions to the standard physical education program at school is the exercise Classics. The introduction of this exercise has had a positive effect in our previous research\cite{5}.

In this study, the effectiveness of using exercise Classics in physical education classes at school is proved. Namely, there was an improvement in indicators not only of children’s coordination abilities, but also improved indicators of the volume of attention of schoolchildren. Thus, the hypothesis that the development of physical abilities has a favorable effect on the development of cognitive and mental processes is confirmed\cite{11-13}.

It is also worth noting the positive impact of the coordination exercise of the Classics in primary school age, which is sensitive for the development of most of the abilities of schoolchildren\cite{10}.

The new study confirms the effectiveness of using a differentiated approach in working with schoolchildren, which ensures individual physical development of the optimal level of each schoolchildren\cite{6,7}.

Conclusion

If at each lesson in physical culture at school, schoolchildren will perform a Classic exercise, then the indicators of not only coordination, but also the volume of attention of children will improve. This will significantly increase the effectiveness of standard physical education lessons at school. The research is relevant and promising for further research in the field of physical education and sports.

Conflicts of Interest: There is no conflict of interest

Source of Funding – Self-funding
Ethical clearance – All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

References


The Possibility of Healing Deep Wounds in Rats Using Helium Neon Laser

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Abstract
Laser treatment is generally used for tissue restoration. Restorative soft tissues include objective, practical and difficult responses.

The goal of the research have being to estimate the irradiation capabilities the helium neon laser irradiation on three different types of wounds depth.

Sixty young male rats were used in this study, divided into three groups. (20 per group) Wounds were created with different depth for the first, second and third group have been irradiated by the laser using He-Ne energies flowing 4 minutes, five out of twenty animals left without radiation is considered to control.

Biopsy was taken from all rats for histological examination at 1, 3 and 7 days after surgery. The results showed a clear enhancement of treatment wounds from control, Helium neon laser failure in the treatment of very deep wounds.

Key words: laser treatment, deep wounds, wound healing

Introduction
Laser therapy is a procedure to obtain the improvement in medical treatment body, acupuncture, dentistry, veterinary practice involves the recruitment and refers to the relationships between the laser and biological tissue radiation monochromatic light in biological tissue to get the effect biomodulative within the soft tissue(1,2)

Investigation effects have varied for various reasons, including the use of different doses of the waves and the use of animal models healthy(3).

It has been improved wound remedial by applying of laser power (4). An assuring analyses for laser to stimulate the healing of ulcers and other wounds on the skin involved(5).

Produces laser radiation modified dynamically, which means that it can stimulate or discourage. So can be laser low dose is ineffective, while the excess energy may prevent healing rather than stimulated. Can treat acute injuries frequently (daily) (6)

Although accelerating tissue healing, the effects of hyperplasia did not appear. During the healing wounds of the laser contained a larger amount of collagen and tensile strength. Therefore, when using health goals, the results may be silent (7).

In spite of several intelligences of certain effects after examinations led in the test center, in animal styles and in medical randomized regulated ordeals, is motionless provocative LLLT(8).

The influence of the laser be contingent on the energy intensity, exposure period and radiated area. The laser wavelength affects the depth of the energy...
Applying low levels of visible light to reduction pain, inflammation and promote wound remedial, tissue and deep nerves, and avoiding soft tissue harm(10).

Studies have shown the number of negative results and positive

The application of laser irradiation technology, He-Ne daily on the wounds till healing has occurred to verify the full deductions at a low level energy by reducing injuries(11).

Researchers emphasized that tissue restoration develops extra active when dealing with low-level laser. They exposes that the laser beam catalyze create of the fibroblast growing element and the frequency these cells. Exposure to chromatic laser radiation faster the remedial procedure, with better collagen fibers increases collagen installation, along per earlier epithelial(12).

Through our previous studies on radiation wound treatment (The study includes the first research of wound healing using laser helium neon confidential 1 cm depth and a length of 1 cm and the second by argon laser) indicating an important speeding in the field dynamic of range OF wound remedial(13, 14), This third paper examines the efficiency of He- Ne laser in treating deep wounds so I dealt with three types of wounds of different depths.

Materials and Method

The use of continuing helium neon laser mechanism made in China type of (Jgq) no. (250) a radiation-point 2 mm in the radius of the wave length from 632.8 nm and power $85 \times 10^{-3}$ W.

Sixty young rats male, age of less than five months, weight approximately of less than fifth hundred gram be there operated applied. Rats have being distributed about three groupings (a), (b), (C). A surface wound 10mm distance be prepared at Thigh all anesthetize rats into all groupings, but the depth was made into (11-15) mm in the first group a, and (15-20) mm second group b, third grouping was performed on (20-30) mm.

All rats in the cage were clean net to prevent pollution.

All Groups (a), (b) and (c) contains (20) rat, fifteen rats exposed using laser helium neion of Energy 0.2 J (Energy Density $= (0.2) / (0.126) = (1.6) J/cm^2$) for 4 min. Another five Non-radioactive animals were considered as a controller grouping. A biopsy of all rats was taken for histological Test analysis at 1, 3 and 7 days after surgery. Tissue Test analysis histopathology be there Conducted for assess the quantity of Inflammatory cells, the appearance of microscopy properties Fibroblasts, Granular tissue plus epithelial cells for every model.

Results

Figure (1): Test analysis histopathology (a) and (b) groupings exposed laser therapy advantage wounding corrective. Indicated a positive reactions began after the firstly post-operative day throughout the decrease from Inflammatory cells by induction Epithelial cells creation after together edges of the injury in comparable the grouping (c) and controller grouping.

On the third day, the consequences of injure remedial were seemed primary on the treated grouping (a, b) in comparly per the treated grouping (c), then controller grouping .The marks designated Inflammatory cells as well as Fibroblasts propagation creation by Fibrous connective soft tissue full in Collagen. Furthermore evident quantity of tissue Granulation be there observed since together edges from incision and Epithelization it was initiated, fig (2). Control Grouping exposed reduction apparition Restorative marks injure, wherever wounding edge full by attacked lifeblood coagulate thru great numeral of Fibroblasts , inflammatory cells permeation by creation a small amount quantity of Epithelial and Granulated tissue after together edges of wound

By the seventh Days after surgery grouping (a), (b) showed Granulated tissue created after Fibroblasts retractor has proliferated, the Receptacles bloody crowded. Within addition to emergence a full restoration of the wound with the restoration of the skin, then there comes about various therapeutic combinations between these groupings. This approved together with another papers. (15)

But it was seen as a Scar tissue in the form of a tinny cover the incision edge then severe hyperplasia in the epithelial cells that come to be larger usual in
the circumscribing soft tissue, was observed and Fibrous connective tissue, cellular in wounding line of controller grouping, fig 3.

But in a very deep wound, it showed the group that received treatment (c) at the 1 be there -3 -7-days after the operation of any reduction in the size of the wound, a very thick blood clot on both sides of the tip. The results showed not any marks of recovery, and a laser he ne failure applying this energy in this style of wounding therapy, fig (4).

**Figure 1.** Firstly days after the operative, comprising grouping (a), (b) slice revealed the edge of wound be fulfilled by blood clot, and explained in Fibroblasts and the spread of blood clotting. Groupings (C) afterward days firstly the slice showed life blood coagulate on the managed location.

**Figure 2.** Grouping (a), (b) slice on the Third day after operation Infiltration made a small number of small inflammatory cells numeral of fibroblasts proliferation and the creation of little fibrous connective tissues. Controller grouping (C) which a blood clot started in the Emergence the beginning of epithelial cells proliferation of fibroblasts with inflammatory cells since together locate.
Figure 3: Grouping (a) a thin layer (b) on the seventh day is the restoration of the completion of the skin including the formation of collagen small connective fibers of connective tissue and not at all inflammable groups in the detection of control showed assembly tissue scar, inflation observed in epithelial cells and connective tissue fibroblasts in the cell line.

Figure 4: Grouping (c) reveal excessive blood clotting for in both locations the wound with a thick edge.

Discussion

In the current paper, the possibility of laser helium neon in the treatment of different depths of wounds.

The development of the Collagen production and prevent inflammation resulted from examination of laser treated tissues.

That guided experience HelumNeion laser (632.8) Nano-meter display rapider stages proliferative inflammatory. Then there was healing great failures in the first stage on the first day of the group (c). That means lacking efficiency in the treatment of a very deep wound (Dogan, SK. et al) and other revealed disadvantage in remedial with laser therapy (16-20). In many animal pattern experiments, lasers have been accepted as a supplement to additional operation in curative treatment, as low-level lasers are used to accelerate wounding remedial (21).
By contrast Status information and medical experiences thru human being may have great results in action laser therapy in wounding restorative\(^{(22)}\).

(Liao X, and Xie GH) who found that healing were completely healed, laser therapy seemed to accelerate the restoration stage of remedial, in which the intensity of the granulation tissue would be pretentious, \(^{(23, 24, 25)}\)

(Ty Hopkins et al) Laser therapy is an active healing to promote partial reduction of wounds from corrosion. Additionally accelerates wounding reduction of non-exposed wounding off the same arm, showing an indirect influence happening the surrounding area tissue \(^{(26)}\).

Numerous hypotheses can benefit clarify the reduction of injuries recover As well In vitro reports have presented an improve in the proliferation of fibroblasts after treatment

**Conclusion**

Laser detection and treatment using long wavelength Helium -neon 632.8 nanometer (1.6) J / cm\(^2\) Energy density exposed for 4 minutes to advance injury to therapeutic incentives in rat, and then Make any benefit results in a very deep wounding using wound related technology.

**Conflict of Interest**: There is no

**Source of Funding**: Self Source

**Ethical Clearance**: Ethical approval of the al-qadisiyah university

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The Role of Legal Sociology in Terms of Covid-19: Large-Scale Social Restrictions (PSBB) in Indonesia

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Abstract

The study of legal sociology is a study that has legal phenomena, but uses social science and sociology theory. The role of Legal sociology is so tight when juxtaposed with a problem that is emerging, namely the global pandemic Coronavirus Disease (Covid)-19. The juridical normative research with a method of approach to the law, conceptual and comparative. Legal materials that have been collected are analyzed by content analysis. Some affected countries that successfully handled the COVID-19 pandemic, including: South Korea, New Zealand, Singapore. Italy and United States containment strategy for handling Covid-19 are contradictory from those countries above. PSBB has been applied in Indonesia and other countries. The PSBB must be accompanied by other programs so that the government can successfully handle COVID-19. PSBB does not guarantee that the community will obey the regulation. The most effective action with all the consequences is regional quarantine or lockdown accompanied by other supporting programs from the government.

Key words: PSBB, Legal Sociology, COVID-19

Introduction

Basic Concepts of the Perspective of Legal Sociology (conceptually and theoretically) there is no single definition related to the perspective of legal sociology. But certainly, the perspective of legal sociology is to understand the reality of law from the perspective of social sciences, especially sociology or in other terms the social science of law. The study of legal sociology is a study that has a legal phenomenon, but uses optical social science and sociological theories. This science is the study of the reciprocal relationship between law and other social phenomena empirically analytic (1)

In essence this is an overall object of the sociology of law so that there is no doubt that a legal system is a reflection of the social system where the legal system was the parts. But the problem is not that simple because it needs to be investigated in the circumstances of how and by how the social system influences the legal system as a subsystem and the extent to which the process of influencing influence is reciprocal (2)

The role of legal sociology is so thick when juxtaposed with the problem that is now emerging, namely the global pandemic Coronavirus Disease (Covid) -19. Every day cases increase, both positive confirmed by COVID-19 infection and death cases. Once the magnitude of the ability of this virus to infect and spread is evidenced by the record that so far there are still many people infected with COVID-19 with increasing numbers. The total number of COVID-19 cases in the world totaled 1,074,290 cases with a death of 56,987 people (3)

Seeing how dangerous COVID-19 is, the social science approach that still prioritizes law as its object becomes very important. In sociology studies, the focal point for COVID-19 is on behavior, practice and institutions. Broadly speaking, it functions to investigate legal phenomena by using the methods and
Theories offered by social science. The use of social science methods in handling COVID-19 involves one main element: Involves serious efforts to overcome a complete perspective through the collection, analysis and interpretation of empirical problems.

The Government, through the Minister of Health, issued regulations in the framework of accelerating the handling of corona virus disease 2019 (COVID-19), namely Permenkes Number 9 of 2020 Concerning Large-Scale Social Limitation Guidelines (PSBB). These actions include restrictions on certain activities of the population in an area suspected of being infected with COVID-19 including restrictions on the movement of people and / or goods for one particular province or district / city to prevent the spread of COVID-19. The restrictions are at least carried out through the consolation of schools and workplaces, restrictions on religious activities, and or restrictions on activities in public places or facilities.

Indonesia has adopted a policy of implementing Large-Scale Social Restrictions which, in principle, is implemented to reduce the widespread spread of COVID-19, based on epidemiological considerations, threat magnitude, effectiveness, resource support, operational technical, economic, social, cultural and security considerations. The policy is in the form of Government Regulation Number 21 of 2020 concerning Large-Scale Social Restrictions in the Context of Accelerating the Handling of Corona Virus Disease 2019 (COVID-19). Seeing the importance of the role of legal sociology with the COVID-19 pandemic conditions, the author is interested in taking up the theme and is associated with the Large-Scale Social Restrictions (PSBB) by the government. The author wants to know whether or not the enactment of these regulations and the very likely impact arising when the regulation is enforced. But in this article we are not considering to compare the law system, economy and demography factors between the countries.

From the explanation above, the writer wants to formulate a problem consisting of: 1). What is the Form of PSBB in Indonesia? 2) What is the form of handling COVID-19 in other affected countries? 3) What is the Role of Legal Sociology in the existence of Large-Scale Social Restrictions (PSBB)? 4). What impacts will occur when the regulation is enforced?

Finding and Discussion

Legal Sociology

Legal sociology, has the object of study of legal phenomena. Legal sociology basically refers to the behavior of individuals and society, as written by Curzon, that Roscou Pound shows the study of legal sociology as a study based on the concept of law as a means of social control. Law in the study of sociology views regulation as a control. Social control here generally means legally questioning whether a behavior is morally good and legally binding. But from a sociological point of view, it can also be viewed in a non-legal form, i.e. simply we can see behavior that is not appropriate “in general” in society as bad behavior.

Coronavirus Disease 2019 (COVID-19)

Coronavirus novel or better known as Corona Virus Disease (COVID) 19 is a disease that first appeared in China in early December 2019. This disease was officially announced on December 31, 2019 which resulted in pain in 41 people with detail 1 (one) person dies. The virus is suspected by WHO as coming from animals which are traded freely in Wuhan, China. One reason is the transmission or spread through bats which are then consumed by humans. This disease quickly spreads throughout China to Europe, the United States, Southeast Asia and Africa. COVID-19 spreads through droplets (particles) that come out through the human mouth when talking to others. This virus can infect anyone if the person is in contact with a positive sufferer COVID-19. Because the level of virulence (strength of the virus causing infection) COVID-19 is so high, contact such as intense communication or long group discussion, risks becoming a place to attach the virus, especially in the mucosal area (tongue, oropharynx, nasal cavity and conjunctiva). Once COVID-19 attaches and eventually infects, if a person’s immunity is low, then that person will very easily experience symptoms of COVID-19.

Strategy Containment of COVID-19 in Indonesia and Other Countries

South Korea
The government through the Prime Minister, the Minister of Health, the Minister of Welfare and the Korean Central and Disease Center, actively communicates and supports one another so that the procurement of Rapid PCR can be produced in mass quantities and quickly and has been approved by the FDA K (Food and Drug Administration) of Korea. The proof is that after the 100th case, Korea can conduct and attach results as many as 1,581 positive cases per day, until finally reaching 11,290 cases in early April (9)

Expanding the range of checks to prevent transmission of contacts in the community includes contact tracing, quarantine and isolation. Koreans have experience in dealing with Pandemics before, namely SARS-CoV, MERS, so with self-awareness to trace contacts from the smallest group by a small group on a massive scale. Tracking the not surrender was emphasized by the government. Openness to one another increases the government’s ability to track down even small districts. In addition, tourists or migrants from outside Korea are not prohibited from entering, but are required to carry out quarantine controlled by the government for 14 days (7) (9)

New Zealand

New Zealand has several steps to deal with COVID-19, namely: 1) Having an approximate modeling of the possibility of a New Zealand population being infected with COVID-19. In New Zealand have used disease modeling to increase these estimates, because modeling can take into account more factors than basic data, including the fact that populations take steps to protect themselves; 2) Elimination System for COVID-19. The government introduced a four-level response system on March 21 and the country was placed on a ‘level 2’ response (which involved restrictions on mass meetings and encouraging increased physical distance). The country then rises rapidly to ‘level 4’ (broadly described as ‘closure’ which involves the closure of all schools, unimportant workplaces, social gatherings and severe travel restrictions) which take effect on the night of March 25, 2020. Emergency national announcements were also announced, giving additional authorities the power to enforce control measures. This approach has a strong focus on border control, which is clearly more easily applied to island nations. It also emphasizes case isolation and contact quarantine to ‘eradicate’ the transmission chain; 3) Border control with high quality quarantine for incoming tourists; 4) Rapid case detection is identified by extensive testing, followed by rapid case isolation, by fast contact tracking and quarantine for contacts; 5) Promotion of intensive hygiene (cough etiquette and hand washing) and the provision of hand hygiene facilities in the public environment; 6) Intensive physical juxtaposition, currently applied as a lockdown (level 4 warning) which includes schools and workplace closures, restrictions on movement and travel, and decisive action to reduce contact in public spaces, with the potential to relax these actions if elimination is successful; 7) A well-coordinated communication strategy to inform the public about control measures and about what to do if they are not healthy, and to reinforce important health promotion messages (10)

Singapore

Singapore Oversight for COVID-19 aims to identify as many cases as possible using complementary detection methods. First, case definitions to identify suspected cases, in health facilities or through contact tracing, are established based on clinical and epidemiological criteria, and evolve over time as more information becomes available. SARS-CoV-2 RT-PCR laboratory testing capacity was rapidly increased to all public hospitals in Singapore and was able to handle 2,200 tests a day for a population of 5.7 million. Likewise, ROK has also rapidly expanded capacity testing, including setting up drive-through testing stations, and has conducted more than 200,000 tests to date. All suspected and confirmed cases were immediately isolated in the hospital to prevent further transmission. Contact tracing was also initiated to determine their history of movement 14 days prior to the onset of symptoms to isolation to determine the possible source of infection and also to prevent further transmission between close contacts (11)

In terms of improving health services, Singapore coordinated by setting up a Network of 800 Public Health Readiness Clinics (PHPC) enabled to improve the management of respiratory infections in primary care settings, with subsidies extended to Singapore residents to provide incentives for them to seek treatment in these PHPCs (9) (11)
In addition to detecting cases and containing spread, prevention of import cases is important to reduce the power of infection of external sources. In Singapore, the temperature and health checks of tourists who came from Wuhan since January 3, 2020, and extended to all travelers since January 29, 2020, have been in place at all ports. Travelers who meet the definition of a suspect’s case are sent directly to the hospital. Singapore on March 4, 2020, has advised Singaporeans to postpone unnecessary trips to mainland China, ROK, Northern Italy and Iran and impose restrictions on entry to visitors from the same area. Returning residents and long-term passport holders with historical travel to the affected areas are subject to 14 days of quarantine (11).

**Italy**

Italy was the first country in Europe to be affected by COVID-19. The government’s unpreparedness to face COVID-19 because many people underestimate this virus. Besides fulfilling to do the test for COVID-19 is only done for people who have symptoms only. Asymptomatic people who are very likely to transmit are not tested. Suburbs in Italy have not been able to facilitate their communities to get the COVID-19 test. The availability of masks is very limited because masks are not produced in Italy and there are not enough ventilators for all patients who need treatment and more than 2,500 hospital beds for patients in intensive care units will be needed in just 1 week to treat acute respiratory distress syndrome that is caused by SARS-CoV-2-pneumonia in Italy (12, 13).

In recent years, health policy has changed a lot in Italy. The number of beds has decreased, and regional autonomy has emphasized inequality in service quality in the national territory. In addition, hospitals may be the main carriers of COVID-19, because they are quickly inhabited by infected patients, facilitating transmission to uninfected patients. Patients transported by our regional system, which also contributes to the spread of diseases such as ambulances and personnel, quickly become vectors (13).

According to many observers, one of the first obstacles in Italy’s response to the outbreak was its failure to recognize the magnitude of the pandemic, and make decisions in real time, when the crisis was taking place. In late February, several Italian politicians, including center-left Democratic Party leader Nicola Zingaretti - who was later infected by COVID-19 - showed themselves drinking in Milan, promoting the idea that the Italian financial center should continue business as usual in the midst virus. To further complicate what is recognized as an unprecedented crisis, Italy has failed to adopt a coordinated approach to emergencies (14).

**United States of America (USA)**

The United States is too slow in responding to the corona virus and has banned travel from Europe, one of several steps officials should have taken earlier to reduce the virus that spreads rapidly. President Trump has repeatedly heralded his decision on February 2, 2020 to stop traveling from China, where the corona virus first appeared in Wuhan city late last year. However, orders that limit travel from Europe do not officially happen until March 11 - one of the government’s biggest mistakes. In the month leading up to the European travel ban, nearly 2 million people from Italy and other countries arrived in the US, according to a new article released by the CDC, which is responsible for disease tracking and prevention. Limited testing and the slow implementation of social distance are also key factors in accelerating cases in the United States, the article continued. Mass gatherings in February, including scientific meetings in Boston, New Orleans annual Mardi Gras celebrations and Georgia funerals, resulted in dozens and dozens of Americans being infected. In addition, more than 100 people traveling on nine separate Nile River cruises in February and early March have come to the US and tested positive for the virus, almost double the number of cases known at the time (15).

**Indonesia**

In the implementation of health outreach in the region, Indonesia has adopted a policy to implement Large Scale Social Restrictions which, in principle, is implemented to reduce the widespread spread of COVID-19, based on epidemiological considerations, threat magnitude, effectiveness, resource support, operational technical, economic considerations, social, cultural and security. The policy is in the form of Government Regulation Number 21 of 2020 concerning Large-Scale Social Restrictions in the Context of Accelerating the Handling of Corona Virus Disease 2019 (COVID-19).
To implement Government Regulation No. 21 of 2020 concerning Large-Scale Social Restrictions in the Framework of Accelerating the Handling of Corona Virus Disease 2019 (COVID-19) (hereinafter referred to as PP No. 21 on PSBB), guidelines for implementing Large-Scale Social Restrictions are needed which regulates more technically regarding the criteria Large-scale Social Restrictions (PSBB). Considering that during the COVID-19 pandemic there were likely to be many people who had been infected or had not yet been detected, or were in the incubation period, so to prevent widespread spread in an area through personal contact, it was necessary to limit large-scale social activities in the area. The limitation of certain activities in question is limiting the gathering of large numbers of people at a particular location.

Normative science of law related to PSBB is very clear, namely to limit citizens in their activities. Empirically found many facts that PSBB does not function as it should. PSBB here is merely a regulation without a detailed explanation related to the implementation of how this PSBB is carried out. The strength of PSBB which is not binding, because the breakdown of regulations only explains the appeal, not the prohibition. All aspects that are restricted such as dismissing schools and workplaces, is an effective way.

The aim of accelerating the handling of COVID-19 in accordance with Permenkes through PSSB is likely to be difficult to achieve. This is because many of the countries that are infected with COVID-19 in large numbers, do something similar to PSBB, namely quarantine or lockdown area to overcome the problem of COVID-19. The lockdown or quarantine area was carried out in Wuhan, China. The quarantine of the area prohibits all individuals from leaving Wuhan or entering Wuhan. All land and air transportation are frozen. There are no flight routes to and from Wuhan. About 9 million people in Wuhan were isolated for weeks. This very strict regional quarantine is able to minimize the spread of infected and dead populations. The case fatality rate for COVID-19 in China is around 3%, one of which is supported by the quarantine of the Chinese government. In addition to quarantine areas, data tracing can also be done. This is because the key to eradicating the COVID-19 virus is that after finding out that there is a positive confirmation, tracing is carried out immediately to anyone who comes in contact with patients.

In view of some PSBB references in several countries, the current Covid-19 handler approach, including in Indonesia, is to conduct PSBB which can lead to regional quarantine. This is because, in the absence of strict regional quarantine rules, even healthy individuals can be transmitted or infected by other individuals. Because Covid-19 can also be transmitted by asymptomatic individuals (carriers or carriers). If lockdowns are not carried out, then what will happen is a buildup of patients in the hospital, while we know the capacity of isolation beds and ventilators in hospitals throughout Indonesia will not be sufficient to handle all patients infected with Covid.

Impact of Large-Scale Social Limits

Although PSBB have not been fully implemented in all regions, there have been many losses in the economic sector that have an impact on the sociological function of money itself, which is driving social change. This is reflected in the increase in termination of employment (layoffs), cut the amount of monthly salary received, and the death of several sectors of the Small and Medium Enterprises (SME) industry. Minister of Manpower Ida Fauziyah revealed that currently there has been an increase in the number of workers laid off. Now the number of workers / laborers / workers laid off and laid off rises to around 1.7 million people.

COVID-19 has long-term negative impacts on society, the health system, the workplace and the individual itself. The social effects of COVID-19 cannot be estimated when it will end. Therefore, it is indeed very necessary long-term planning to anticipate the social and economic consequences that will be caused.

Conclusion

Many countries have also implemented systems such as the PSBB and some have even implemented such as national quarantine or lockdown. Of the several successful countries, PSBB and regional or national quarantine cannot stand alone, but other supporting programs are needed such as the country’s ability to conduct mass Rapid PCR tests on its citizens; the ability to track patient contacts accurately and continuously; encouragement and enthusiasm to meet
adequate facilities such as ventilators and Personal Protective Equipment (PPE); and economic assistance for affected communities; must be run synergistically. Synchronization of central and local government regulations to achieve the above is needed so that the handling and eradication of COVID-19 to be effective and maximum.

**Conflict of Interest:** The authors declare that there is no conflict of interest.

**Source of Funding:** This study received no external funding but was financially supported by the authors (ourselves).

This research is a task project for research and development for subjects in the field of legal sociology, in the master of law program, postgraduate University of Muhammadiyah Malang (UMM), academic year 2019-2020 under Prof. Dr. Rahayu Hartini, S.H., M.Si., M.Hum. by using independent funds.

**Ethical Clearance:** All procedures followed were in accordance with the ethical standards of the responsible committee on.

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Factors affecting the Occurrence of Tuberculosis Destroyed Lung

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Abstract

Introduction: Pulmonary Tuberculosis (TB) disease remains a public health. In recent years, the prevalence of TB has increase. Dangerous complication of Pulmonary TB can destroy lungs. In 83.3% cases of destroyed lung, the patient are found to have a history of pulmonary tuberculosis. Objective: The aimed of this study was to identify factors affecting the occurrence of Tuberculosis Destroyed Lung in Lampung Province, Indonesia in 2017. Method: This study is an analytic observation with a case control design. The sample were obtained through total sampling. Primary and secondary data were obtained from interview and medical records. The samples were taken from 64 patients in several hospitals in Lampung, Indonesia. Results: The results showed the relationship between age with destroyed lung (p = 0.897), gender with destroyed lung (p = 0.511), education level with destroyed lung (p = 0.754), occupation with destroyed lung (p = 0.060), income with destroyed lung (p = 0.482), cigarette smoking with destroyed lung (p = 0.013), and the presence of multiple diseases with destroyed lung (p = 0.748). Conclusions: There is a significant relationship between Cigarette Smoking to the incidence of destroyed lung.

Keywords: Tuberculosis Lung, Destroyed Lung, cigarette smoking

Introduction

Tuberculosis (TB) is an infectious disease caused by an infection of the bacterium Mycobacterium tuberculosis. Six countries lead 60% of new cases which are India, Indonesia, China, Nigeria, Pakistan and South Africa. Worldwide, the rate of decline in the incidence of TB was 1.5% in 2014-2015. In this case it is necessary to intensify 4-5% of annual decline by 2020 to reach the first indicator of the TB settlement Strategy. The World Health Organization (WHO) reported that about one third of the world’s population has been infected with M. tuberculosis, but only about 5-15% of the 2.3 billion peoples infected will develop active TB disease. It was estimated that in 2015 there were 10.4 million new cases of TB in the world, of which 5.9 million (56%) were men, 3.5 million (34%) were women, and 1 million (10%) were children. In addition around 1.4 million people died of TB in 2015. Although the death rate from TB decreased by 22% between 2000 and 2015, TB was still in the top 10 causes of death in the world in 2015 (¹). The number of cases reported is a number that shows the number of new patients found and recorded among 100,000 residents in a particular area. This number is useful for showing a tendency in increase or decrease findings in the region. The number of notifications of the new cases of bacteriologically confirmed pulmonary tuberculosis in 2015 in Indonesia amounted to 74 per 100,000 population. It decreased compared to 2014 which amounted to 77 per 100,000 population. meanwhile the incident of all tuberculosis cases in 2015 amounted to 130 per 100,000 population, it increased compared to 2014 which amounted to 129 per 100,000 population (²).

The incidence of all tuberculosis cases in Lampung Province in 2015 was 105 per 100,000 population, ranked the lowest 6 of all provinces in Indonesia and also ranked the highest with treatment success rates of 95.2% according to the standard set by WHO 85% (³). One of the complications of Pulmonary Tuberculosis is Destroyed Lung. A study in India showed that 83.3% of cases of pulmonary lesions has a history of...
pulmonary tuberculosis (3). Pulmonary tuberculosis in advanced conditions can cause progressive, extensive and irreversible destruction of pulmonary parenchyma and damage to pulmonary function (4).

Pulmonary tuberculosis in sustainable conditions can cause progressive, extensive and irreversible destruction of pulmonary parenchyma and damage to pulmonary function (4). The term of destroyed lung is usually used to describe damage to the pulmonary parenchyma caused by the sequelae of pulmonary TB that occurs for many years, and is caused by chronic airway obstruction. Radiology can be found in a picture of shrinkage of lung volume, the presence of cavities, bronchiectasis and fibrosis. The response of the fibrosis network can make retraction of the hilum and mediastinum so that it shifts towards damaged lung tissue. Meanwhile other lung parts that are still good compensate for being large (5). Until now, no treatment guidelines were available for patients with pulmonary TB (4). There are various factors that influence the incidence of pulmonary tuberculosis such as; age, sex, education level, occupation, income, cigarette smoking and the presence of multiple diseases (6-8). The researchers aim to determine the factors that influence the incidence of pulmonary tuberculosis in Lampung Province in 2017.

**Methods**

This study is analytical with case control an approached. This research was conducted at the Bandar Lampung, Indonesia (Dr. H. Abdul Moeloek Hospital, Dr. A. Dadi Tjokrodipo Hospital and Harum Melati Clinic Pringsewu). Time of study was February-March 2017. The sample size was 34 patients with destroyed lung and 30 patients with pulmonary TB as controls. The data of this study are primary and secondary from direct interviews and medical records. Bivariate analysis was done using a Chi square test with p < 0.05.

**Results**

Figure 1a above shows the distribution of clinical diagnosis of 64 patients, in which the number of patients with destroyed lung was 34 (53.1%) and patients with pulmonary tuberculosis is 30 (46.9%). Figure 1b shows that the distribution based on the age of 64 patients in which the number of patients with destroyed lung was 21 (61.8%) in productive age and 13 (38.2%) in unproductive age. It also shows the comparison of patients with pulmonary TB of 19 (63.3%) in productive age and 11 (36.7%) in unproductive age.

Figure 2a shows the distribution of patients by gender of 64 patients in which the number of patients with destroyed lung was 22 (64.7%) male and 12 (35.3%) female. It also compares patients with pulmonary TB of 17 (56.7%) male and 13 (43.3%) female. Figure 2b shows the distribution of patients based on education level from 64 patients in which the number of patients with destroyed lung was 25 (73.5%) in low level education category and 9 (26.5%) in high level education category. It also compares patients with pulmonary TB of 21 (70%) in low level education category and 9 (30%) in high level education category.

Figure 3a shows the distribution of patients based on occupancy of 64 patients; in which the number of patients with destroyed lung was 1 (2.9%) unemployed and 33 (97.1%) employed. It compares patients with pulmonary TB of 5 (16.7%) unemployed and 25 (83.3%) employed. Figure 3b shows the distribution of patients based on income from 64 patients in which the number of patients with destroyed lung was 33 (97.1%) with low income and 1 (2.9%) with high income. It compares the patients with pulmonary TB of 28 (93.3%) with low income and 2 (6.7%) with high income.

Figure 4a shows the distribution of patients based on cigarette smoking from 64 patients in which the number of patients with destroyed lung was 23 (67.6%) regular smoker and 11(32.4%) lifelong non smokers. It compares patients with pulmonary TB of 11 (36.7%) regular smoker and 19 (63.3%) lifelong nonsmoker. Figure 4b shows the distribution of patients based on the presence of multiple diseases from 64 patients in which the number of patients with destroyed lung was 3 (8.8%) accompanied by other diseases and 31 (91.2%) not accompanied by other diseases. It compares patients with pulmonary TB of 2 (17.7%) accompanied by other diseases and 28 (82.3%) not accompanied by other diseases. Details of statistical analysis can be seen in table 1.
Table 1. Analysis of the Relationship between Pulmonary Tuberculosis and Destroyed Lung

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>9.35</td>
<td>0.34 – 2.58</td>
<td>0.897</td>
</tr>
<tr>
<td>Gender</td>
<td>1.402</td>
<td>0.51 – 3.84</td>
<td>0.511</td>
</tr>
<tr>
<td>Education Level</td>
<td>1.190</td>
<td>0.40 – 3.54</td>
<td>0.754</td>
</tr>
<tr>
<td>Occupational</td>
<td>0.152</td>
<td>0.02 – 1.38</td>
<td>0.060</td>
</tr>
<tr>
<td>Income</td>
<td>2.357</td>
<td>0.20 – 27.39</td>
<td>0.482</td>
</tr>
<tr>
<td>Cigarette Smoking</td>
<td>3.612</td>
<td>1.28 – 10.15</td>
<td>0.013*</td>
</tr>
<tr>
<td>Presence of Multiple Diseases</td>
<td>1.355</td>
<td>0.21 – 8.71</td>
<td>0.748</td>
</tr>
</tbody>
</table>

*Significant < 0.05

Figure 1. (A) Frequency distribution of clinical diagnosis; (B) Frequency distribution of the age.

Figure 2. (A) Frequency distribution of the gender; (B) Frequency distribution of the education level.
Discussions

It can be concluded that there was no significant relationship between age and destroyed lung. However, the mean age was 55.6 years old. A number of patients with pulmonary TB were in the age of 15-55 years old. It is influenced by the relationship of the age group related to employed in the outside environment and without their awareness in contact with a patient with pulmonary regarding TB germs are transmitted through the air (8).

It can be concluded that there was no significant relationship between sex with patients with destroyed lung. Unrelated results can be caused by the small number of the sample. The incidence of destroyed lung in male is more than in female. It is due to the fact that the number of male smokers number was high and they also have a poor behavior to do self prevention from the transmission of TB germs (8).

It can be concluded that there is no significant relationship between the education level of patients and destroyed lung. Unrelated results can be caused by the small number of the samples. Knowledge is the basis of taking prevention and treatment of tuberculosis. Ignorance of the community will hinder attitudes and actions towards the prevention and eradication of pulmonary TB disease as a sick person so that it can eventually become a source of transmission and spread of pulmonary TB disease to people who are around him. The study of Rohayu et al, with the title of analysis of risk factors for positive smear pulmonary TB incidence in coastal communities at Kadatua Health Center in South Buton Regency in 2016 showed no relationship between knowledge (education level) and incidence of pulmonary TB (p = 0.018) (9).

It can be concluded that there is no significant relationship between the patient with destroyed lung and occupation. Unrelated results can be caused by the small number of the samples. The majority of case patients were unemployed. If a patient is unemployed, it will affect the utilization of health services. One’s work will also be able to reflect the small amount of information received. That information will affect
someone in making decisions to utilize existing health services, to provide nutritious food, to keep a healthy home environment and to maintain health status. This can have an effect on the body, spiritually, and socially so that if these needs are not met, it can reduce the health status where the immune system decreases so that it is susceptible to pulmonary TB \(^{(10)}\).

It can be concluded that there is no significant relationship between the patient with destroyed lung and income. Unrelated results can be caused the small number of samples. In the study of Kurniasari et al in pulmonary TB, it is stated that the lack of economic status causes them not to have the ability to make a healthy or fulfilling house, to obtain health information, to get access to health services and to fulfil nutrition which in turn results in low endurance so it’s easy to get an infection \(^{(11)}\).

It can be said that patient with pulmonary TB on cigarette smoking are 3,612 times more at risk of developing destroyed lung. The limitations on air flow in patients with pulmonary tuberculosis are most patently caused by smoking. Indoor air pollution from cigarette smoking can increase the risk of severe infection and lung damage. Cigarette smoking contains more than 4,500 chemicals that have various toxic effects, genital and carcinogenic muta \(^{(12)}\). These substances have a proinflammatory and immunosuppressive effect on the immune system of the respiratory tract. Therefore it can increase the risk of destroyed lung. This study is similar with Sayuti on patient with pulmonary TB stating that smoking in the home is associated with the incidence of pulmonary TB in East Lombok Regency with P-Value = 0.0163 and factors can increase the incidence of TB disease due to indoor air pollution \(^{(13)}\).

It can be concluded that there is no significant relationship between the patient with the destroyed lung and the presence of multiple diseases. Unrelated results can be caused by the small number of samples. The results of this study were similar to the research of Izzati et al which concluded that diabetes mellitus was not associated with the incidence of pulmonary TB in the Andalas Community Health Center work area with p-Value = 0.186 \(^{(14)}\).

**Conclusion**

Based on the data taken, it can be seen that the incidence of pulmonary tuberculosis from 64 patients was 34 (53.1%) have destroyed lungs. Those with destroyed lung were 21 (52.5%) of productive age, 22 (56.4%) male, 25 (54.3%) with low education level, 33 (56.9%) employed, 33 (54.1%) low income, 23 (67.6%) smoker, and 31 (52.5%) Not accompanied by other diseases. There was no significant correlation between age, gender, education level, occupation, income and presence of multiple diseases with destroyed lung in Lampung Province, Indonesia in 2017. A significant association was found between patient with destroyed lung with cigarette smoking.

**Acknowledgments:** We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

**Conflict of Interest:** The authors declare that they have no conflict of Interest.

**Funding:** None

**Ethics Statement:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

**References**


Health Rights for Indonesian Migrant Workers in Malaysia: A Legal Perspective

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Abstract

Work is an application of the human being’s responsibility in existing. The type of work may be chosen freely whether it is within the country or abroad. This is a normative law research which is supported by empirical study. The normative law study is used to analyze the constitutional regulations regarding the health rights of the Indonesian migrant workers. Meanwhile, the empiric law study is used to analyze the implementation of the regulations. The health right implementation of the Indonesian migrant workers in Malaysia is applied through health insurances based on the sosco regulations. The payment of the health rights for the Indonesian migrant workers is the responsibility of the employer. The research results show that in the aspect of health, the work agreements do not go according to the contract. The health security of the Indonesian migrant workers in Malaysia is still not effective as it only covers accidents within the working hours.

Keywords: Health Rights, Indonesian Migrant Workers, Law

Introduction

Every person has the right to work and to obtain wages. They also have the right to be treated fairly in working relations as written in the Republic of Indonesia’s 1945 Constitution. The legal protection for the guarantee of adequate living through working opportunities for the citizens have a grand sense in achieving a state’s success. Thus, as mentioned by Izziyana (1) it is an obligation for the government to achieve that right.

Work is an application of the human being’s responsibility in existence. Work may be chosen freely, whether it is in the country or overseas. The state has the obligation to provide work for their citizens well. According to Arinanto (2), it must also bring positive influence for life survival of the citizens without discrimination.

Indonesia is one of the countries with the highest rate of exported migrant workers, as said by Payaman. (3) It may be from the demand of the hosting country or the initiative of the migrant worker outsourcing company overseas, according to the Center of Research and Information Development, the National Body for Indonesian Migrant Worker Placement and Protection. (4)

One of the sectors which has the power to dynamize the economy of Asian countries is the sending of migrant workers, as said by Azmy. (5) The guarantee on legal protection for migrant workers is crucial. According to Agusmidah (6) in line with the increasing interests for becoming Indonesian migrant workers, the rate of inhumane treatments towards the migrant workers has also increased. Cases which are related to the life of the migrant workers become more variative. Worse yet, said Rusli (7) it is now developing towards human trafficking which is a category of violation of the human rights.

The protection of health rights for the migrant workers is a crucial problem. This is because if the Indonesian migrant workers experience health
problems, it will automatically inhibit their abilities in working. It will create a negative impact towards their working relations, which will also impact their wages. The problems faced by the migrant workers are also global problems of humanity. This is because in it, there are various potentials of problems which must be acted upon by the world, starting from human trafficking, black market, fake documents, crime, and also economic problems, according to Calderon. (8) The economic problems are the cause of the international migration of workforces, said Birca. (9)

Nowadays, the sending of migrant workers is also a motive of crime perpetrators in trafficking people, which will lead to the unfulfillment of the health guarantees in the hosting country, as opined by Nuraeny. (10) The cases which happened at that time pushed the government to issue a policy which stops the sending of migrant workers and some certain jobs to Middle Eastern countries on 2015. This is because, said Flambonita, (11) the government considers the aspects of safety, human rights, equal distribution of working opportunities, the fulfilment of workforces according to the demand, and also legal protection.

Indonesian migrant workers are prone to harassment and exploitation. Such fragility causes the migrant workers’ rights to be easily ignored by some parties, such as the outsourcing agent, the employers, and the hosting countries, according to Krustiati. (12) Indonesian migrant workers mostly have low education. Thus, their knowledge and skills are limited, including their knowledge on their rights for health. Migrant workers may bring negative impacts socially, culturally, politically, and economically, thus the government needs to take some actions for anticipation, as said by Kassim in Johari. (13)

The Constitution No. 18 of 2017 regulates the protection on the Indonesian migrant workers comprehensively. The said regulation only places the migrant workers in their hosting countries. It also applies the regulations on foreign workforces, where in it also applies the social security system or the insurance which protects the foreign workers, with the regulation on a written agreement between the hosting country and the government of the Republic of Indonesia as the sending country, said Toruan. (14) Social security is a form of social protection as the rights for the employees regarding the job they have, as opined by Manea. (15) These requirements have the aim to reach legal protection for the Indonesian migrant workers. The focus of this research is to describe the protection of the health rights guarantee for the Indonesian migrant workers in Malaysia through the legal perspective.

**Research Method**

This research is a legal normative study which is supported by empiric research. The method of research is the legal normative method which is used to analyze some constitutional regulations which are applied regarding the health rights of the Indonesian migrant workers. Meanwhile, according to Fajar and Ahmad (16), the legal empiric research method is used to analyze the application of that regulation, whether or not it is applied well, and to describe the problems which arise due to the issuing of that regulation.

**Results and Discussion**

The hosting and the protection of workers overseas is actually related to the relations between countries. Thus, it is clear that the government have the power to manage the placement and the protection of the migrant workers overseas. The government cannot act by themselves. Thus, it must include the roles of the provincial government, the city/regency government, and the private institution.

The sending of the migrant workers overseas have contributions in the social and in the developmental aspects. It may increase the state’s foreign currency. It will open up new working opportunities. It will ease the pressure of problems. The society may experience obtaining high wages, and it may decrease unemployment. If we try to see the sense and the aim of sending Indonesian migrant workers overseas in a wider context, it will not only solve the employment problems in Indonesia, but it is also a form of economic improvement.

Employment Act and the Workman Compensation’s Act regulates the guarantee for working and living appropriateness of the Indonesian migrant workers, under the surveillance of the Department of Workforces. Starting from 2019, the both domestic and non-domestic migrant workers in Malaysia will obtain guarantees from
the Social Security Organization/Sosco. So far, the Sosco social security was only meant for the local workers, as mentioned in a conversation with Ahmad Dahlan (Staff of the Republic of Indonesia’s Workforce Penang, Division of Immigration, on February 26th, 2018). This policy has the aim to fulfil the standardization so that it is according to the ILO convention, which stated that the constitution on workforces must be universal and uniform.

The Indonesian migrant workers in Malaysia are regulated in the Workmen’s Compensation Act 1952 and the insurance policies paid by the employers. The Workmen’s Compensation Act 1952 will soon be revoked, and it will be changed with the Sosco regulation which has a higher beneficial impact. The discourse proclaimed the obligation to pay RM14 to RM15 a month to Sosco to protect the health security aspect of the workers. The workers will obtain much benefit as they are protected by an insurance and they may obtain a life-long compensation. The employers have the responsibility to pay for the Sosco. Socso owns one of the best hospitals in Southeast Asia in Melaka. If there are migrant workers who experienced an accident, they may obtain full treatment until healed, as said by Neni Kurniaty (Staff of the Republic of Indonesia’s Workforce Penang, Executor of Counsellor Function II, on February 26th, 2018).

The health aspect of the migrant workers is not really given attention in the work contract. In reality, sick leave may only be obtained after a written permit from the employer, and that will only happen if they are granted permission, as said by Khazinah, an Indonesian migrant worker in Malaysia, in a conversation (February 24th, 2018). Just like in the case of Nirmala Bonet, the attorney said that there is a point in the work contract regarding health security. Yet, that is no different from social security given from compensations. To obtain compensation from the case of torture, it took ten years, in a conversation with Anis Hidayah (Head of the Center for Migrant Study and Research, April 9th, 2018).

Fringe benefits may also be forfeited if the worker takes sick leave, as mentioned in a conversation with Rubiatun (an Indonesian migrant worker in Malaysia, February 24th, 2018). This shows that there is no difference between sick leave and annual leave. Health security is deemed as not effective enough as it only covers the physical health of the migrant workers who experienced accidents in the working hours. In the view of legal protection theory, the Malaysian policies has not shown enough care for the health of the migrant workers.

**Conclusion**

In the aspect of health security, the migrant workers will obtain much benefits as they are protected by the insurance. They may also obtain life-long compensation based on the Sosco regulation. The payment to sosco becomes the responsibility of the employers. The health aspect of the migrant workers is not really taken care of in the work agreements. Sick leave may only be obtained after a permission from the employee. Fringe benefits may also be forfeiter if the worker still takes this sick leave. This shows that there is no difference between sick leave and annual leave. Health security is deemed as not effective enough as it only covers the physical health of the migrant workers who experienced accidents in the working hours.

**Ethical Clearance:** Yes

**Conflict of Interest:** No

**Source of Funding:** Universitas Muhammadiyah Surakarta

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Comparison of Clinical Characteristics and Neuroimaging of Cerebral Palsy with and without Epilepsy in Children

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Abstract

Background: Cerebral palsy (CP) is one of the main causes of limited activity in children. The prevalence of CP ranges from 2.6 to 2.9 per 1000 live births. Most of children with CP have at least one comorbid, including epilepsy. Epilepsy in CP is difficult to control, and can increase the severity of motor impairment and cognitive function, therefore the prognosis is poor. The incidence of epilepsy in CP is associated with a specific clinical profile. This study aimed to analyze the comparison of clinical characteristics of cerebral palsy in children with and without epilepsy.

Methods: A cross-sectional study was conducted using medical record of children with cerebral palsy in pediatric neurology outpatient clinic in Dr. Soetomo Hospital Surabaya in March - May 2020. Children with CP who met the inclusion criteria were included in this study. The variables studied included sex, perinatal history (preterm birth, low birth weight, and neonatal asphyxia), neonatal seizures, spastic type, level of GMFCS, head circumference, neuroimaging features, hearing loss, and eye abnormalities. The study subjects were divided into two groups. Group 1 consisted of children with CP and epilepsy. Group 2 consisted of children with CP without epilepsy. Data analysis was performed using the Chi-square test and fisher’s exact test using SPSS.

Result: Significant comparison of the characteristics were found in the history of neonatal seizures and the level of GMFCS. The percentage of neonatal seizures was higher in group 1 at 61.3% (p=0.049). The degree of GMFCS in group 1 was dominated by GMFCS III while in group 2 it was dominated by GMFCS IV (p=0.047). Subjects with GMFCS I and II levels were only found in group 2, while in group 1 with higher level of GMFCS, they were GMFCS III, IV, and V. More abnormal neuroimaging was found in group 1, namely 64.3%, while in group 2 it was 57.1%. There was no statistically significant difference of neuroimaging characteristics between the two groups (p= 0.911).

Conclusion: There were differences in clinical characteristics associated with neonatal seizures and GMFCS between CP with and without epilepsy.

Keyword: Cerebral palsy, epilepsy, neuroimaging.

Background

Cerebral Palsy is a chronic disease affecting the center of movement control with clinical manifestations that are visible in the first few years of life and generally do not get worse at later ages. Based on data from NSCH and NHIS, it was reported that the prevalence of CP ranged from 2.6 to 2.9 per 1000 live births¹.
Children with CP suffer from motor problems and other disorders, such as intellectual problems, seizures, behavioral and emotional disorders, speech and language disorders, as well as eye and hearing problems. Previous studies stated that epilepsy is most often found in children with spastic CP. Another study states that 39% of spastic CP in children have epilepsy. The spastic types most often found in children with CP are hemiplegia, diplegia, and quadriplegia. In several studies, it is stated that the most common type of spastic in CP with epilepsy is quadriplegia.

Epilepsy is a major prognostic factor in mental and motor function in children with CP. Gross Motor Function Classification System is a classification of children’s motor abilities and limitations at home, school, and in the environment. GMFCS IV-V levels were found more frequently in CP accompanied by epilepsy. Whereas in CP without epilepsy, the GMFCS level that was mostly obtained was level I-II.

Head circumference abnormalities, namely microcephaly, is one of the most common clinical manifestations of spastic CP patients. Another study states that microcephaly is more common in CP patients with epilepsy, and microcephaly increases the risk of epilepsy in CP.

Other co-morbidities that often accompany CP patients are hearing loss and eye disorders. Children with CP had hearing loss in 10%, and 2% were bilateral deaf. Another study states that nearly 20% of children with CP also have eye disorders, such as strabismus, amblyopia, nystagmus, cortical blindness, and visual abnormalities. Vision screening needs to be performed on children with CP so that these abnormalities can be detected early. Children with CP and epilepsy are more likely to have abnormal brain imaging. Brain atrophy was also reported more frequently in CP with concomitant epilepsy and reached statistical significance in one study. The abnormality of the imaging features in CP is controversial. This study aimed to analyze the comparison of clinical characteristics and neuroimaging of cerebral palsy children with and without epilepsy.

Methods and Materials
A cross-sectional study was conducted using medical record of children with cerebral palsy in pediatric neurology outpatient clinic in Dr. Soetomo Hospital Surabaya in March - May 2020. Children with CP with epilepsy and without epilepsy who met the inclusion criteria were included in this study. Children with multiple congenital anomalies, genetic syndromes, and incomplete medical record (age, sex, body weight, head circumference, perinatal and postnatal history, spastic type, level of GMFCS) were excluded. The study subjects were divided into two groups. Group 1 consisted of children with CP and epilepsy. Group 2 consisted children with CP without epilepsy.

The variables studied included sex, perinatal history (preterm birth, low birth weight, and neonatal asphyxia), neonatal seizures, spastic type, level of GMFCS, head circumference, neuroimaging features, hearing loss, and eye abnormalities.

Data analysis was performed using the Chi-square test and Fisher’s exact test with a significant significance value of p <0.05. All statistical analyzes were conducted using SPSS version 25.

Result
There were 50 subjects included in this study. The characteristics of the subjects were showed in table 1.

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>n=50</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year) (mean ± SD)</td>
<td>5,66 ± 2,84</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>40%</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>60%</td>
</tr>
</tbody>
</table>
In this study, the characteristics were compared between cerebral palsy with epilepsy and without epilepsy included sex, spastic type (quadriplegia, diplegia, and hemiplegia), level of GMFCS, perinatal history, neonatal seizures, head circumference, neuroimaging features, hearing problem, and eye abnormalities. Neuroimaging examination was performed on 42 of the total study subjects. ENT examination was performed on 41 of the total subjects. The comparison of these characteristics was described in table 2.
<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=31</td>
<td>n=19</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (38,7)</td>
<td>8 (42,1)</td>
<td>1.000*</td>
</tr>
<tr>
<td>Female</td>
<td>19 (61,3)</td>
<td>11 (57,9)</td>
<td></td>
</tr>
<tr>
<td>Preterm birth</td>
<td>9 (29,0)</td>
<td>8 (42,1)</td>
<td>0.522*</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>8 (25,8)</td>
<td>7 (36,8)</td>
<td>0.611*</td>
</tr>
<tr>
<td>Neonatal asphyxia</td>
<td>12 (38,7)</td>
<td>7 (36,8)</td>
<td>1.000*</td>
</tr>
<tr>
<td>Neonatal seizure</td>
<td>19 (61,3)</td>
<td>7 (36,8)</td>
<td>0.049*</td>
</tr>
<tr>
<td>Spastic type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemiplegia</td>
<td>1 (3,2)</td>
<td>1 (5,3)</td>
<td>0.885**</td>
</tr>
<tr>
<td>Diplegia</td>
<td>10 (32,3)</td>
<td>7 (36,8)</td>
<td></td>
</tr>
<tr>
<td>Quadriplegia</td>
<td>20 (64,5)</td>
<td>11 (57,9)</td>
<td></td>
</tr>
<tr>
<td>Level of GMFCS</td>
<td></td>
<td></td>
<td>0.047**</td>
</tr>
<tr>
<td>GMFCS I</td>
<td>0</td>
<td>2 (10,5)</td>
<td></td>
</tr>
<tr>
<td>GMFCS II</td>
<td>0</td>
<td>2 (10,5)</td>
<td></td>
</tr>
<tr>
<td>GMFCS III</td>
<td>12 (38,7)</td>
<td>4 (21,1)</td>
<td></td>
</tr>
<tr>
<td>GMFCS IV</td>
<td>9 (29)</td>
<td>8 (42,1)</td>
<td></td>
</tr>
<tr>
<td>GMFCS V</td>
<td>10 (32,3)</td>
<td>3 (15,8)</td>
<td></td>
</tr>
<tr>
<td>Head circumference</td>
<td></td>
<td></td>
<td>1.000**</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>15 (48,4)</td>
<td>10 (52,6)</td>
<td></td>
</tr>
<tr>
<td>Normocephaly</td>
<td>14 (45,2)</td>
<td>7 (36,8)</td>
<td></td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>2 (6,5)</td>
<td>2 (10,5)</td>
<td></td>
</tr>
<tr>
<td>Neuroimaging features (n=42)</td>
<td>n=28</td>
<td>n=14</td>
<td>0.911*</td>
</tr>
<tr>
<td>Abnormal</td>
<td>18 (64,3)</td>
<td>8 (57,1)</td>
<td></td>
</tr>
<tr>
<td>Hearing examination (n=41)</td>
<td>n=25</td>
<td>n=16</td>
<td>1.000*</td>
</tr>
<tr>
<td>Hearing problem</td>
<td>4 (16)</td>
<td>3 (18,7)</td>
<td></td>
</tr>
<tr>
<td>Ophthalmology examination (n=41)</td>
<td>n=25</td>
<td>n=16</td>
<td>1.000*</td>
</tr>
<tr>
<td>Eye abnormalities</td>
<td>5 (20)</td>
<td>3 (18,7)</td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square; **Fisher’s exact test
Significant comparison of the characteristics were found in the history of neonatal seizures and the level of GMFCS. The percentage of neonatal seizures was higher in group 1 at 61.3% (p=0.049). The degree of GMFCS in group 1 was dominated by GMFCS III while in group 2 it was dominated by GMFCS IV, where there was a significant difference for the clinical characteristics of GMFCS (p = 0.047). Subjects with GMFCS I and II levels were only found in group 2, while in group 1 with higher GMFCS level, they were GMFCS III, IV, and V (Table 2).

In this study, data on EEG examination results were reported only in children of CP with epilepsy. There were 70.9% subjects with abnormal EEG and 29.1% subjects with normal EEG. The most common type of EEG abnormality was sharp waves (16.1%).

Neuroimaging examinations in the form of head MRI and head CT scan were performed on 42 subjects. More abnormal neuroimaging was found in group 1, namely 64.3%, while in group 2 it was 57.1% (p=0.911). The description of neuroimaging abnormalities found in the two groups is presented in Table 3.

### Table 3. Comparison of the neuroimaging features of cerebral palsy children with epilepsy and without epilepsy

<table>
<thead>
<tr>
<th>Neuroimaging features</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain atrophy</td>
<td>5 (17.8%)</td>
<td>4 (28.6%)</td>
</tr>
<tr>
<td>Periventricular leucomalacia</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Cystic encephalomalacia</td>
<td>1 (7.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>4 (14.3)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Infarct</td>
<td>2 (7.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Schizencephaly</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Subdural hygroma</td>
<td>1 (3.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ventriculomegaly</td>
<td>1 (3.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Infection</td>
<td>2 (7.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Calcification</td>
<td>2 (3.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Normal</td>
<td>10 (35.7)</td>
<td>5 (42.9)</td>
</tr>
</tbody>
</table>

### Discussion

In the study of all subjects with cerebral palsy, the percentage was higher in the cerebral palsy group with epilepsy, which was 62%. A study conducted found that 89.9% of cerebral palsy patients developed epilepsy. A meta-analysis study showed the prevalence of epilepsy in CP children of 36% - 62%. There is a close relationship between epilepsy and cerebral palsy. Epilepsy is considered to be one of the most common
Neurological disorders that accompany patients with CP. It is estimated that about 20% of cases of childhood epilepsy are the result of brain lesions that also cause CP. The CP patients in this study were predominantly female, both in the CP group with epilepsy and without epilepsy, namely 61.3% and 57.9%, respectively, and there was no significant difference between the two groups \( (p = 1,000) \). This result is different from the results of another study that male sex dominated both in the CP group with epilepsy and the CP group without epilepsy with a percentage of 57.6% and 60.3% respectively, but there was no significant difference between the two sex-related group \( (p = 0.673) \).

In the perinatal history, the characteristics assessed include preterm birth, low birth weight infants, and neonatal asphyxia. In preterm birth, the percentage was higher in the CP group without epilepsy, namely 42.1% compared to the CP group with epilepsy, which was 29.0% \( (p = 0.522) \). Previous study stated that 20.9% of patients with CP were diagnosed with preterm birth, with most details in the CP group with epilepsy (21.6%) compared to the CP group without epilepsy \( (44.7\%) \)\(^{11} \). Other study reported that a higher frequency of epilepsy in term infants with CP similar to this study, but there was no correlation was found between gestational age and risk of epilepsy in other studies\(^{21} \).

In the characteristics of low birth weight babies, a higher percentage was also found in the CP group without epilepsy (36.8%) compared to the CP group with epilepsy (25.8%) with \( p = 0.611 \). This is also in line with research conducted by El-Tallawy et al., Which did not get a significant difference in terms of birth weight in the two groups\(^{4,12} \).

Neonatal asphyxia was found in 38% subjects. The percentage was more in CP with epilepsy (38.7%) compared to CP without epilepsy (36.8%) and there was no significant difference with \( p = 1,000 \). In a previous study the highest percentage of low apgar scores was in the CP group with epilepsy compared to the group without epilepsy \( (24.4\% \text{ vs } 6.9\%) \) with \( p<0.02 \)\(^{21} \). Asphyxia is a condition that can cause the brain to go into hypoxia, ischemia, and hypercapnia, which can cause brain damage. Brain damage causes CP at a later date and when it hits certain areas such as the cerebral cortex and temporal lobe, it will cause epilepsy\(^{22} \).

Neonatal seizure was found in 52% subjects, and most were found in the CP group with epilepsy (61.3%) compared to the CP group without epilepsy (36.8%) with \( p = 0.049 \). A study was found that there were more patients with neonatal seizures in the CP group with epilepsy than those without epilepsy \( (38.1\% \text{ vs } 17.2\%) \) and there was a significant difference with a value of \( p = 0.000 \)\(^{12} \). In line with the study it was found that neonatal seizures in CP with epilepsy compared to CP without epilepsy were 48.4% vs 7.9% \( (p<0.004) \)\(^{19} \). Some literature states that neonatal seizures increase the risk of death and the occurrence of neurological sequelae in neonates in the form of epilepsy. In various previous studies it was reported that neonatal seizures are a risk factor that plays a role in the occurrence of epilepsy in CP patients\(^{3,18} \). Widiastuti’s research conducted at 3 teaching hospitals in Jakarta found a neonatal seizure mortality rate of 47.4%. Neonatal seizure mortality is related to the degree of clinical condition of the neonate after birth. This study did not find subjects who had neonatal seizures in the CP group with epilepsy. This may be related to the high mortality in neonatal seizures\(^{23} \). Neonatal seizures result in intrinsic lesions in the brain that have the potential to produce epileptogenesis, and epileptogenesis is a process of neural tissue that will develop into recurrent epileptic seizures\(^{24} \). In addition, neonatal seizures will produce more extensive brain damage, and when it hits certain areas such as the cerebral cortex and temporal lobe, it will cause epilepsy\(^{22} \).

In this study, when compared between the CP group with and without epilepsy, it was found that in the two groups the most common types were quadriplegia \( (64.5\% \text{ vs } 57.9\%) \), then diplegia \( (32.3\% \text{ vs } 36.8\%) \), and hemiplegia \( (3, 2\% \text{ vs } 5.3\%) \) with \( p=0.885 \). In line with another study mentioned that the CP group with epilepsy and without epilepsy were both dominated by the spastic quadriplegia type, but the percentages between the two were quite different \( (58.3\% \text{ vs } 34\%) \) with \( p <0.05 \)\(^{4} \). Cerebral palsy with spastic quadriplegia is common in patients who have a high incidence of seizures, ranging from 50 to 94%, which, in turn, may be a reflection of the severity of damage to the brain\(^{25, 26} \). Another study states that the most common type of CP with epilepsy is the type of quadriplegia, which causes the most severe motor disorders and involves all extremities and is associated with mental retardation due to brain damage that is relatively greater than the type of CP without...
In this study, the highest level of GMFCS in all study subjects was GMFCS IV. In the CP group with epilepsy, there were no GMFCS levels I and II, only GMFCS levels III, IV, V. In the CP group without epilepsy, all GMFCS degrees were obtained. The level of GMFCS describes the severity of motor disturbances in CP. Some previous studies have reported that GMFCS degrees IV and V are dominated by CP patients with epilepsy. The same results were also obtained in a study, where GMFCS grade IV-V was found the most in the CP group with epilepsy and grade I-II was mostly found in the CP group without epilepsy, and there were significant differences between the two groups. These studies are in line with this study that obtained a high degree of GMFCS which illustrates the severity of the dominant motor function found in CP accompanied by epilepsy.

The literature states that there is a straight relationship between epilepsy and the degree of motor impairment, as well as a relationship with mental disorders. Epilepsy is the main prognostic factor in mental and motor function in children with CP.

In this study, microcephaly is slightly more common in the CP group without epilepsy compared with epilepsy (50% vs 48.4%) but there was no statistically significant difference between these two groups. In previous study was found the distribution in the CP group with epilepsy and without epilepsy were almost the same. Another study states that microcephaly increases the risk of epilepsy in CP. According to Wibowo and Saputra, in their research, the incidence of head circumference is inversely proportional to the incidence of preterm gestational age and low birth weight. This suggests that head circumference abnormalities in cerebral palsy may occur in the post-natal period, which is caused by disorders when brain development has not been completed, namely at the age of less than 3 years and the most likely cause in this study is CNS infection. According to literature, CNS infection can cause head circumference abnormalities by destroying brain mass causing microcephaly.

Of the 41 study subjects, 17.07% were found with hearing loss, and 16% were found in the group with epilepsy and 18.7% in the group without epilepsy. There was no statistically significant difference between the two groups (p = 1,000). Research conducted by Delacy et al. mentioned as many as 12% of research subjects with CP have hearing loss. Research conducted by Zafeiriou et al., obtained hearing loss as much as 19.1% in the CP group with epilepsy and 12.7% in the CP group without epilepsy. Although it is different from this study which states that hearing loss is more common in the CP group without epilepsy, there is no statistically significant difference between the two groups.

Approximately 12% of children with CP experience hearing loss. The incidence of hearing loss is higher when the etiology of CP is associated with low birth weight, kernicterus, neonatal meningitis, or hypoxic-ischemic encephalopathy. Hearing loss is generally a neurogenic abnormality in high-pitched perception, making it difficult for children to grasp words. In addition, CP children are prone to recurring chronic ear infections which will impair their hearing. Hearing loss also has a negative impact on language development. CP children with intellectual disabilities and abnormal radiological examinations are at high risk for hearing loss. Hearing loss is often diagnosed late, and research shows that more than half of children with severe hearing loss are diagnosed when the child is almost 3 years old.

In the CP group with epilepsy, there were 20% with eye abnormalities and 18.7% in the CP group without epilepsy. There was no significant difference between the two groups regarding eye abnormalities. Research conducted by Zafeiriou et al., found 19.1% eye abnormalities in the CP group with epilepsy and 12.7% in the CP group without epilepsy. The eye disorders in this study were refractive errors, strabismus, and decreased vision. Although the study found a significant relationship between eye disorders and epilepsy, there is no data in the literature on the relationship between eye problems and epilepsy.

Neuroimaging in this study was performed using head CT scan and head MRI. There were 64.3% of the neuroimaging features were abnormal in the CP group with epilepsy, and 57.1% in the CP group without epilepsy and there were no significant differences between the two groups. In line with the study conducted by Senbil et al., It was stated that the abnormal neuroimaging picture was found as much as 74.2% in the CP group with epilepsy and 48.8% in the CP group without epilepsy.
and there was no significant difference between the two groups. The most neuroimaging features found in the two groups were brain atrophy. Other types of disorders found are maldevelopment, encephalomalacia, ventriculomegaly, periventricular leucomalacia, cystic lesion, hydrocephalus, and cerebral infarction. The same results were obtained in this study. Most of the cases with cerebral atrophy are the end result of prenatal or perinatal ischemia globally with extensive neurological damage possibly due to seizure. The effects of imaging abnormalities on CP are controversial. In one study, MRI abnormalities were recorded in 86.7% of patients, and abnormal findings on MRI did not significantly affect epilepsy progression and seizure outcome.

**Conclusion**

There were no differences in sex, perinatal history (prematurity, LBW, neonatal asphyxia), head circumference, spastic type, hearing loss, eye abnormalities, and neuroimaging features between CP with and without epilepsy. There were differences in clinical characteristics associated with neonatal seizures and GMFCS between CP with and without epilepsy.

**Conflict of Interest**: None declared.

**Source of Funding**: The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

**Ethical Clearance**: Approved by researched ethical committee Dr. Soetomo General Hospital Surabaya No. 1916/KEPK/III/2020.

**References**


Association of rs865429 C/T polymorphism in SOST gene with Coronary Heart Disease in Iraqi Type 2 Diabetes Mellitus Patients

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Abstract

Sclerostin, encoded by the SOST gene, It has also been shown that sclerostin is expressed in aortic VSMC (vascular smooth muscle) and upregulation of SOST gene has inhibitory effects on the aortic aneurysm and atherosclerosis development. Aims: The current study aims to explore potential the association between SOST gene single nucleotide polymorphisms (rs865429 C/T) and coronary artery diseases (CHD) in type 2 diabetes mellitus (T2DM) patients, in addition to the effect of this SNP on the level of serum sclerostin and other glycemic parameters. Material and methods: From the Iraqi population, we enrolled 300 T2DM patients (150 T2DM with CHD and 150 T2DM without CHD). Serum blood glucose, serum insulin, HbA1C, and sclerostin were estimated. Genotyping for rs865429 C/T in SOST gene was achieved by RFLP (polymerase chain reaction-restriction fragment length polymorphism). Results: T2DM patients having CT + TT genotype included in the studied were at increased risk for CHD in T2DM (odd ratio: 0.4444 CI: 0.2800 to 0.7054) and related to a high serum sclerostin level in comparison with type 2 diabetic patients with CC genotype. Conclusions: Type 2 diabetic patients with T allele who have elevated plasma concentrations of sclerostin are at high risk for coronary artery diseases.

Key words: single nucleotide polymorphism, polymerase chain reaction restriction fragment length polymorphism, type 2 diabetes mellitus, coronary artery diseases.

Introduction

Worldwide, the cardiometabolic risk is increased, and it is the leading cause of mortality and disability, such as impaired lipid and glucose metabolism, high blood pressure, obesity, and systemic inflammation. Also, these metabolic features are present in many individuals with T2DM, which may contribute to the approximate doubling of coronary heart diseases (CHD) risk in persons with diabetes. Sclerostin is a glycoprotein (190 amino acids) is the major antagonist of the Wnt- pathway. Sclerostin especially binding to the LRP 5/6 complex inhibits Wnt signalling and lowering β-catenin translocation into the nucleus, as well as a reduction in cholesterol uptake. Osteocytes are a major source of sclerostin, liver, though chondrocytes, kidney, and avascular wall (aorta) may also secrete it. The relationship between Wnt inhibitors (DKK1 and sclerostin) and arterial wall calcification and cardiovascular events is unclear. Higher serum level of sclerostin is observed in T2DM patients when compared with healthy subjects, and serum sclerostin level is correlated positively with the diabetes duration, and glycated haemoglobin (HbA1c). Sclerostin has been reported to have positive negative or no correlation with arterial calcification. Even its connection with cardiovascular events is controversial. Sclerostin, encoded by the SOST gene, It has also been shown that sclerostin is expressed in aortic vascular smooth muscle and upregulation of SOST gene has inhibitory effects on aortic aneurysm and atherosclerosis development. This study is intended to evaluate the association between rs865429 C/T in SOST gene and coronary artery diseases in T2DM patients.
Experimental

Study Design

Three hundred patients (150 Women, 150 Men) age 57.7 ±1.64 years, with a diagnosis of T2DM according to the American Diabetes Association criteria. Subjects, including 150 T2DM patients with CHD (T2DM-CHD), and 150 T2DM patients without CHD (T2DM). The coronary heart disease patients were selected from patients in the catheterization ward of Najaf Center for Cardic Surgery, at Al-Sadder Medical City, Najaf, Iraq (with more than 70% stenosis in each of the main coronary vessels). The protocol of the work was approved by the Ethics Committee of College of Science at Kufa University. Also, each subject signed an informed approval before participating in the study. From the patients, written informed consent was obtained. All patients were Iraqi, without congenital heart disease, cardiomyopathy, severe liver or kidney disease. Patients with vasculitis, connective tissue diseases and familial hypercholesterolemia were also excluded.

Biochemical analysis

At the same time of the day, blood samples were taken for all subjects and after 12-hour fasting. Blood samples collected in two tubes. The first was left to allow clot formation for 10 min, and then centrifuged within 20 min at 3000 Xg, and sera were separated. The reminder blood samples were collected in the tubes containing K₂EDTA for measurement percentage of glycated haemoglobin (HbA1c). The percentage of Hemoglobin A1c was measured by a colorimetric method by using the HbA1c kit (Stanbio, USA). Serum insulin and sclerostin levels were determined using a solid phase enzyme-linked immunosorbent assay kits (Elabscience, USA).

Genotyping analysis

The genomic DNA extraction kit (Promega, USA) was used to extract genomic DNA from the peripheral blood, concerning manufactures guidelines. Genotyping for the studied SNP was accomplished using polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP). The primer sequences of rs865429 C/T SNP were present in table 1, and the restriction enzymes name and length of the restriction fragments were presented in table 2.

Table 1: Primer sequence for rs865429 C/T SNP in SOST gene

<table>
<thead>
<tr>
<th>SNP in SOST gene</th>
<th>Primer sequence (5 to 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs865429C/T</td>
<td>Forward primer: CAGGAGGTGAACCCCCAGCTCGAAGGGG</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: AGGCAAGGGTTGGGACTGGGGTGGCTGCT</td>
</tr>
</tbody>
</table>

Table 2: Restriction enzyme name and length of the restriction fragments.

<table>
<thead>
<tr>
<th>SNP in SOST gene</th>
<th>Restriction enzymes</th>
<th>Cut Temperatures</th>
<th>Amplification length</th>
<th>Restriction fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs865429C/T</td>
<td>Ncol</td>
<td>37</td>
<td>365bp</td>
<td>365,102,263</td>
</tr>
</tbody>
</table>
Statistical Analysis

The present study data are expressed as mean ± standard deviation (Mean ± SD). The two groups parameters were compared used students \( t \)-test. All statistical analyses were performed using the statistics is a powerful statistical software platform (SPSS) software (V20;0 IBM Corporation, Armonk, NY. USA). Calculated probability (p-value) less than 0.05 was considered significant. Hardy-Weinberg equilibrium and Proportions of genotypes of alleles were tested using the standard \( \chi^2 \) test, in addition to odds ratios (ORs) and 95% confidence intervals (CI) were calculated.

Results and Discussion

The demographic data and clinical of all participants are existing in table 3. As point out in table 3, the levels of HbA1c and fasting insulin were significantly higher in the T2DM with CHD when compared with T2DM without CHD (\( P < 0.0001 \)). HOMA-IR in T2DM-CHD patients was elevated significantly (11.111 ± 3.617) in comparison to the pathological control group (27.672 ± 9.519). Moreover, T2DM-CHD pointed out a significant increase in the level of sclerostin compared to the non-CHD diabetic pathological control group (181.357±10.959, and 154.285±12.377 Pg/ml, respectively) (\( p \leq 0.0001 \)).

Table3: Hemodynamic characteristics and Clinical of participants and comparison of Sclerostin in the studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>T2DM-CHD</th>
<th>T2DM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>75 Female/75Male</td>
<td>75 Female/75Male</td>
<td></td>
</tr>
<tr>
<td>Age years</td>
<td>56.617± 8.128</td>
<td>57.167± 7.764</td>
<td>0.549</td>
</tr>
<tr>
<td>T2DM duration</td>
<td>30.063± 5.216</td>
<td>29.969±1.996</td>
<td>0.829</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>241.390± 79.586</td>
<td>253.048±92.963</td>
<td>0.243</td>
</tr>
<tr>
<td>HbA1C %</td>
<td>9.381± 1.445</td>
<td>7.373±1.779</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum insulin [µIU/ml]</td>
<td>36.539± 8.566</td>
<td>27.672± 9.519</td>
<td>0.0001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>11.111 ±3.617</td>
<td>6.668 ±2.901</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sclerostin ( Pg/ml)</td>
<td>181.357±10.959</td>
<td>154.285±12.377</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Sclerostin gene was amplified for the detection of rs865429C/T in the patients involved in this study. The amplicon size of the amplification product was 365bp. Then it was digested by Neol restriction enzyme. The digestion products were analyzed by 2% agarose gel electrophoresis. Patients with the wild type homozygote CC exhibited 365bp uncut fragment. While, patients with the of heterozyote CT type showed three bands with sizes of 365bp, 102bp, and 263bp. Those with the homozygote TT type yielded 2 fragments of 102bp, and 263bp fragments (Figure 1).
Figure 1: The PCR product of rs865429C/T SNP in SOST gene analyzed by agarose gel electrophoresis. Lanes A: for patients with the wild type homozygote CC genotype that revealed one fragment with the size of 365bp. Lane B: for patients have the heterozygote GT genotype exhibited three fragments with sizes of 365bp, 263bp, and 102bp. Lanes C: for individuals has the mutant homozygote TT of 253bp, 102bp.

The genotyping of the studied polymorphism in T2DM patients are seen in table 4. Data indicated no significant difference between the observed and expected genotypes. The genotyping results of the studied SNP were found to be consistent with Hardy-Weinberg equilibrium.

Table 4: Hardy-Weinberg equilibrium analysis of rs865429C/T SNP in SOST gene.

<table>
<thead>
<tr>
<th>Results</th>
<th>Genotyping number</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>Observed</td>
<td>29</td>
<td>71</td>
</tr>
<tr>
<td>Expected</td>
<td>27</td>
<td>126</td>
</tr>
</tbody>
</table>

To evaluate the gene-disease associations for the investigated SNP, different inheritance models were considered. The selection of the most appropriate model was achieved via the estimation of the odd ratio. The statistical analysis of the genotype distribution of rs865429C/T SNP in the SOST gene under various inheritance models significant differences under co-dominant and recessive models, when the data of T2DM-CHD and T2DM groups were compared. The results were found to be associated with the recessive and dominant models for the rs865429C/T SNP in the SOST gene. Allele association of rs865429 SNP was analyzed for the relevance with the risk of development of coronary heart diseases in diabetic patients. The Results indicated a frequency of the C allele of 36.33% and 23.33% and the T allele of 63.67%, 76.67% in T2DM-CHD and T2DM groups respectively with an insignificant difference (Table 5).
Table 5: Analysis of rs865429C/T Genotype Association with Coronary Heart Diseases under Different Inheritance Models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotype</th>
<th>Frequency %</th>
<th>Odd ratio (CI 95%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T2DM</td>
<td>T2DM-CHD</td>
<td></td>
</tr>
<tr>
<td>Co-dominant</td>
<td>CC</td>
<td>19</td>
<td>10</td>
<td>1.3380 (0.5737 - 3.1209)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>71</td>
<td>50</td>
<td>2.8500 (1.2396 - 6.5523)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>60</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>CC</td>
<td>19</td>
<td>10</td>
<td>0.4925 (0.2209 - 1.0982)</td>
</tr>
<tr>
<td></td>
<td>CT + TT</td>
<td>131</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Recessive</td>
<td>CC + CT</td>
<td>90</td>
<td>60</td>
<td>0.4444 (0.2800 to 0.7054)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>60</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>0.3633</td>
<td>0.2333</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.6367</td>
<td>0.7667</td>
<td></td>
</tr>
</tbody>
</table>

The biochemical characteristics T2DM-CHD patients were analyzed in relevance to the genotype of the selected SNP under the recessive model. For the rs865429C/T SNP in the SOST gene, the recessive model included patients with genotypes CC+CT vs TT. The analysis was carried out with the use of the student’s t-test. The analyses of the data of the rs865429C/T SNP in SOST gene indicated significant elevations (P=0.0001) of levels of fasting blood glucose, fasting blood insulin, HbA1c, and HOMA-IR, in patients have CT+TT genotypes when compared with those of CC genotype (Table 6). Data of sclerostin concentration revealed a significant elevation (P=0.0001) in patients who have TT genotypes in comparison with those of CC+CT genotype.

Table 6. Differences of biochemical characteristics in relevance to the genotypes of rs865429C/T SNP in the SOST gene of T2DM-CHD patients under the recessive model.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CC+CT (60)</th>
<th>TT(90)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>216.193± 98.104</td>
<td>283.720 ± 76.810</td>
<td>0.0001</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>7.984± 2.029</td>
<td>10.002 ± 2.779</td>
<td>0.0001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>26.930 ± 8.566</td>
<td>37.982 ± 6.910</td>
<td>0.0001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>9.987±4.819</td>
<td>14.944 ±3.012</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sclerostin (pg/ml)</td>
<td>131.402 ±19.878</td>
<td>194.006±28.298</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
The current study assesses the relationship between rs865429C/T single nucleotide polymorphism in the SOST gene with CHD risk in T2DM. Interestingly, in our population, the selected SNP data show similarity with Hardy-Weinberg equilibrium. Statistically, a significant association was observed between the TT genotype with the risk of CHD in T2DM. Iraqi carriers of the TT genotype seem to be more susceptible to the disease. The pooled odd ratios of multiple comparisons of genotyping models, co-dominant, dominant, and recessive were 2.963 (0.906-4.8366, P=10-4), 0.4925 (1.2396- 6.5523, P=0.0834), and 0.444(0.2800- 0.7054, P= 0.0006), respectively. To realize the impact of SOST gene polymorphism (rs865429 C/T) in the development of CHD in type 2 diabetics, we have to focus on mechanisms by which Sclerostin is engaged.

The GWA has proved that SOST gene is one of the hub genes that play a role in osteoporosis pathology. While, Deveci et al.; reported that SOST gene rs865429C/T influences weight and body mass index of Turkish women. And, Pifers et al.; evaluated SOST rs10534024 polymorphism on 783 young and 600 elder Danish men. They reported an interaction between body shape and SOST rs10534024 polymorphism. We also observed an association between CHD in T2DM and rs865429C/T polymorphisms in the SOST gene. T2DM is a major cardiovascular disease risk factor. There are numerous certificates that both genetic and environmental factors contribute to this risk. Gene risk factors identified for cardiovascular diseases is crucial to understand the aetiology of the diseases. Although, mechanisms of atherogenesis in large part stay to be defined. Observational researchers have explained that the progression of the disease involves crisscross between immune cells with both endothelial cells and VSMCs. The fundamental cells inside the media layer of arteries are VSMCs and are important to maintaining the arterial wall integrity of arteries. They take part in the remodeling of the arterial wall and have serious roles in atherosclerosis through all disease stages. The VSMCs display marked plasticity in response to lipoprotein accumulation, vascular injury, and inflammation during progression of disease through re-programming gene expression, i.e., phenotype switching, a shift to a proliferative, promigratory, and activated phenotype. There is rising evidence on the extraskeletal functions of sclerostin, highlighting to its role in many vascular disorders. Recent studies have pointed that under calcifying conditions, the VSMCs are able of producing a phenotypic transition to osteoblast-like cells, under calcifying conditions, which are capable of expressing the typical bone markers, like sclerostin.

Various researchers notified the link between sclerostin level and vascular tissue calcification. Besides the contribution of sclerostin in some disorders associated with processes of vascular calcification, it is controversial in the mechanism by which sclerostin can affect the calcification process, some of them propose that sclerostin has a protective role, while others propose the opposite. In aortic tissue, sclerostin is expressed in VMSCs. While, in atherosclerotic plaques which obtained from carotid endarterectomy, sclerostin is detectable by immunohistochemical staining in VMSCs as well as in macrophages.

It is reasonable to suggest here that this SNP is an important genetic marker that predisposes the occurrence of CHD in type 2 diabetics. Hence, more prospective research should be performed to provide the use of sclerostin and rs865429 SNP in the SOST gene as a good biomarker to identify an increased predisposition to coronary heart diseases in type 2 diabetic Iraqi patients.

**Conclusion**

The present work describes the rs865429C/T SNP in the SOST gene encoding sclerostin in a T2DM patient with CHD. The mutation has a devastating effect on the biological function of sclerostin by increasing extracellular sclerostin levels, thereby, its antagonistic activity on canonical Wnt signalling contributes to the disease phenotype. Also, The association between CHD and rs 865429C>T polymorphism in the SOST gene may open new insights on pharmacogenetics studies of CHD in T2DM.

**Limitation:**

Our study has a limited number of T2DM patients; events might have restricted the power of our study to detect statistically significant associations.

**Acknowledgment:** we are grateful to all the participants.

**Funding Sources:** self.
Conflicts of Interest: Declared none.

Ethics Statement: This experiment was approved by the Central Committee for Bioethics in college of Sciences/ Kufa, Iraq.

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1458 Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1


Knowledge of HIV Transmission and Factors Related to the Incidence of HIV/AIDS in Adolescents in Indonesia

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Abstract

Adolescents are vulnerable to various health risk, especially caused by social interactions, like juvenile delinquency and drug abuse, premarital sex and unwanted pregnancy to an increased incidence of sexually transmitted infections including HIV/AIDS. The number of AIDS cases in students is 282 cases, more than double prostitutes who are 137 cases. The purpose of this study is to find out how is the knowledge of young people about HIV/AIDS, how HIV are transmitted and what are the factors that associated with HIV incidence in adolescents in Indonesia. This paper uses a qualitative method with literature study or library research, then data obtained is analyzed using descriptive analysis methods. HIV/AIDS can be transmitted through bodily fluids contact and is related to several factors such as gender, family factors, community environment, technology and communication. Cooperation of all sectors, parents, social institutions, and society to support government programs for HIV/AIDS-free Indonesia.

Key words: Transmission, HIV, AIDS, adolescents, Indonesia

Introduction

Adolescents in both men and women experience puberty which is maturation very quickly both physically, psychically and socially in preparation for maturity. One of the characteristics of teenagers is having a high curiosity, liking challenges and adventures, starting to find yourself and daring to risk what is done without thinking long. Based on these traits, if the youth do not have strong faith, do not make decisions or the youth are too free without proper control back from the family or the environment then the youth will very easily fall into a bad thing that can damage their morals and future (1).

Adolescents are derived from the Latin word Adolescentia which means “to grow” or “to grow into adulthood”. Children considered to be adults have been able to perform or conduct reproductive activities (2). When it’s about determining age to define youth, some sources have different age restrictions but the differences aren’t far off. According to the WHO, teenagers are residents aged 10 -19 years. Teenagers are residents aged 13 - 20 years. While according to National Population and Family Planning Board (NPFP), teenagers are residents aged 10 - 21 years. Thus, adolescents are a younger generation or population that is approximately 10 -21 years old and is going through puberty or transitioning from childhood to maturity to be ready to reproduce (3).

Adolescence is a critical phase in the formation of teenage characters in both male and female adolescents as a result of changes in each individual. Physical changes in some parts of the body, psychic changes and ways of thinking up to social and environmental changes have a huge impact on adolescents. Teenagers who are unable to control themselves will easily get carried away by currents that can damage the morale and future of adolescence. Based on this, problems in adolescents are complex and interconnected problems, ranging from large numbers, social problems to health problems (4). The number of youth population in Indonesia every year is increasing. According to Ministry Of National
Development Planning, Central Bureau of Statistic and NPFP, in 2015 the youth population was 66.0 million and in 2016 increased to 66.3 million or about 25% of the total population of 258.7 million (5). This means that one in four people in Indonesia is a teenager. A large proportion of teenagers have their own problems that cannot be ruled out, one of which is social and health problems.

Adolescence is the age of self-search that allows teenagers to connect with peers. Friends and the environment have a profound effect on the condition of adolescents, especially in social lives that have an impact on health. Adolescents tend to experience increased susceptibility to various health risk threats, especially those caused by poor social interactions, ranging from adolescent delinquency and drugs abuse, sexual and reproductive health problems such as premarital sex and out-of-wedlock pregnancies to an increase in the incidence of Sexual Trafficking (STI) diseases including HIV/AIDS (6).

HIV or Human Immunodeficiency Virus is a virus that attacks the human immune system. People with HIV will experience a decrease in the immune system so that it is easily infected with diseases including AIDS (Acquired Immuno-Deficiency Syndrome). The trend of HIV incidence in Indonesia tends to increase every year. In 2014, HIV positive cases in Indonesia totaled 32,711 cases, in 2016 as many as 41,250 cases and 2018 as many as 46,659 cases. As of 2018, the number of HIV positive cases in Indonesia reached 327,282 cases, while AIDS cases reached 114,065 cases. The highest percentage of HIV-positive cases by age group was in the productive age group of 25 - 49 years old by 70.4% followed by adolescents 15 - 24 years old by 18.2%. According to the work, the number of AIDS cases in schoolchildren/students is 282 cases, more than double the number of sex offenders in 137 cases (7).

UNICEF noted that the trend in the number of teen deaths aged 10-19 from HIV/AIDS worldwide was 71,000 adolescents in 2005 and increased to 110,000 in 2012 and there were 2.1 million adolescents in the world living with HIV/AIDS in 2012. The death rate or Case Fatality Rate (CFR) due to HIV/AIDS from year to year tends to decrease. In 2018 CFR AIDS in Indonesia amounted to 1.03%, based on this description, researchers are interested in studying adolescents’ knowledge of HIV/AIDS, HIV transmission and factors related to the incidence of HIV/AIDS in adolescents in Indonesia (8).

**Methods**

This writing uses qualitative methods with the type of writing of literature studies. Data and information are obtained from data libraries or scientific research. In retrieving data or information must ensure that the source taken throughout its contents can be held accountable for its truth and validity.

**Results**

HIV/AIDS disease in the world, especially in Indonesia, is a complex problem. The cause of the occurrence of the disease is strongly related to environmental factors, namely social influence and physical social interaction. Transmission of HIV/AIDS disease from sufferers to others can occur through the exchange of bodily fluids. HIV that enters the body will destroy CD4 cells. CD4 cells are part of white blood cells that serve to fight infection. The fewer CD4 cells in the body, the weaker the immune system, so sufferers will be easily exposed to or contracted other diseases.

<table>
<thead>
<tr>
<th>Adolescent knowledge of HIV/AIDS</th>
<th>IDHS 2012</th>
<th>IDHS 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Male</td>
<td>84.7%</td>
<td>86.1%</td>
</tr>
<tr>
<td>- Female</td>
<td>89.0%</td>
<td>91.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HIV/AIDS prevention methods (intercourse sexual), Using condoms</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Male</td>
<td>63.1 %</td>
<td>58.2 %</td>
</tr>
<tr>
<td>- Female</td>
<td>66.5 %</td>
<td>50.8 %</td>
</tr>
</tbody>
</table>
Restrict sexual intercourse in one partner who is not infected with HIV and does not have another partner
- Male
- Female

Based on the above data, adolescent knowledge that restricting sexual intercourse to only one partner who is not infected with HIV and does not have another partner can prevent HIV transmission increases by 4.8% in men and 27.8% in women.

Table 2. Knowledge of HIV transmission from mother to child

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IDHS 2012</th>
<th>IDHS 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother-to-child HIV transmitted during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>61.0%</td>
<td>63.1%</td>
</tr>
<tr>
<td>- Female</td>
<td>74.3%</td>
<td>76.1%</td>
</tr>
<tr>
<td>Mother-to-child HIV transmitted during childbirth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>52.5%</td>
<td>55.2%</td>
</tr>
<tr>
<td>- Female</td>
<td>63.7%</td>
<td>67.1%</td>
</tr>
<tr>
<td>Mother-to-child HIV transmitted during breastfeeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>61.1%</td>
<td>61.5%</td>
</tr>
<tr>
<td>- Female</td>
<td>73.7%</td>
<td>75.5%</td>
</tr>
</tbody>
</table>

The above data shows that knowledge of the transmission of HIV from mother to child during pregnancy, childbirth and breastfeeding increased in both male and female. The most likely cause of HIV transmission in adolescents is the contact of bodily fluids in the form of blood, semen and vaginal fluids during intercourse. This relates to juvenile delinquency and free association. Many teenagers enter the world of drugs, especially teenage boys. In addition, free sex and prenuptial sex are also increasingly becoming among adolescents so that the incidence of HIV in adolescents is increasing.

Table 3. A teenager’s comprehensive knowledge of HIV/AIDS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IDHS 2012</th>
<th>IDHS 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>11.6%</td>
<td>12.8%</td>
</tr>
<tr>
<td>Female</td>
<td>13.0%</td>
<td>15.6%</td>
</tr>
</tbody>
</table>

Source: IDHS 2012, IDHS 2017
Table 4. Knowledge of Voluntary HIV Counseling and Testing (VCT)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IDHS 2012</th>
<th>IDHS 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6.3%</td>
<td>32.2%</td>
</tr>
<tr>
<td>Female</td>
<td>11.0%</td>
<td>48.8%</td>
</tr>
</tbody>
</table>

Source: IDHS 2012, IDHS 2017

The above data shows that there is a comprehensive increase in knowledge in both women and men about HIV/AIDS (knowing that condom use is consistent during sexual intercourse and having only one faithful partner who is not infected with HIV can reduce the risk of HIV transmission as well as rejection of misunderstandings such as mosquito bites and sharing food with HIV sufferers can transmit HIV).

Discussions

HIV/AIDS disease not only occurs in adults and the elderly but can also attack newborns, children and adolescents. The large incidence of HIV in adults indicates that the sufferer contracted in adolescence and began to be detected in adulthood, because the incubation period of HIV takes years. That’s not surprising considering that teenagers are starting to have a lot of friends from different backgrounds and will have a lot of relationships or interact with people. Then from such interactions will have an influence on the adolescent individual, can be a positive influence as well as a negative. Negative influences that can’t be controlled by teenagers can lead teenagers into the world of free association and impact adolescent delinquency including acts of drug abuse, free sex and leading to HIV infection. Adolescent knowledge of HIV/AIDS is a fundamental aspect of HIV transmission prevention efforts.

HIV/AIDS is transmitted through the exchange or entry of infected bodily fluids. Bodily fluids that can be a medium of HIV transmission include:

a. Sperm and vaginal fluids

HIV transmission can occur through sexual intercourse, both homosexual and heterosexual. HIV transmission can occur through intercourse both vaginally, anally and orally. People who have intercourse with a changing partner have a greater risk of contracting HIV.

HIV prevention programs are focused on three aspects of behavior, namely condom use, restricting sexual intercourse to only one partner who is not infected with HIV and has no other partner, and delaying first-time sexual intercourse.

b. Blood

Contact between open wounds, blood transfusions and the use of syringes can transmit HIV. The use of syringes that can transmit HIV is interchangeable use, syringes should be used in sterile and disposable conditions only. But usually users of narcotics injectable (composer) and tattoo maker will use the syringe alternately. In addition, medical patients such as doctors or nurses who are impaled by syringes after use by patients with HIV can also transmit HIV.

c. Mother-to-child transmission

HIV can be transmitted from a mother with HIV-positive status to her child or called Mother to Child Transmission. Transmission can occur in both pregnancy, childbirth and breastfeeding through breast milk. Transmission occurs through the mother’s blood that is directly exposed to her child during pregnancy, the labor process, and through breast milk during lactation.

Various factors related to the incidence of adolescent HIV in Indonesia are largely influenced by the social environment. These factors include:

a. Gender

Teenage boys are more likely to experience HIV incidence than teenage girls. Teenage boys tend to be more daring to perform risky sexual behaviors such as risky courtship behaviors even to have sexual
intercourse by changing partners. In addition, the social environmental factors that are quite influential in teenage boys are free association and drug abuse. Many drug users use syringe media interchangeably to insert substances into the body (composer). The use of syringes is what causes the contracting of HIV.

b. Family

Families especially parents are the closest neighborhood to teenagers. Tatara and family education is the first education that forms the character of youth. Adolescents who have good control and are supervised for their association have a lower risk of developing HIV. In addition, the wholeness of the family also has a connection to the incidence of HIV in adolescents. Teenagers from broken home families are more likely to get HIV because they tend to be stressed and more free to get along.

c. Community Environment

The environment has a considerable connection and influence on the incidence of adolescent HIV/AIDS. The community environment in this case starts from the scope of the school, peers, groups / communities / organizations and the community in general in the environment around the youth residence. Peer and group or community influences are most prone to HIV/AIDS-risk behaviors so desperately need more attention. The scope of the school includes a safe environment due to the monitoring and supervision of the school, especially teachers, during the scope and hours of the school.

d. Technology and Communication

Increasingly today the development of technology is getting more sophisticated especially mobile phones. Advanced technology is like a double-edged knife for teenagers. On the one hand, it makes it easy to access knowledge and information around the world, making it easy to gain insights. But on the other hand, the misuse of smartphone and internet technology by teenagers can have an adverse impact. Teenagers can freely access anything including pornography sites that can then influence his sexual behavior. Coupled with social media that facilitates communication often abused teenagers to transact illicit goods such as drugs for consumption. The wrong way around seems facilitated by the ease of internet access on smartphones. Freedom without control in adolescents will have an impact on free association and juvenile delinquency that can increase risky behaviors for contracting HIV.

According to the description above, teenagers who live freely without control from both themselves and others will be very easily plunged into bad things that can damage the morale and future of adolescence. Especially in adolescents who have less knowledge about things related to adolescents will be more concerned about their future, including the possibility of having an HIV/AIDS disease.

Knowledge of HIV status as early as possible can be a method of preventing HIV transmission in people with HIV-negative status and effective coping or health care for people with HIV-positive status. HIV testing and counseling service called Voluntary HIV Counseling and Testing (VCT) is a service for early detection of HIV and access to supportive therapy, opportunistic infectious therapy and anti-retroviral therapy (ART) for both HIV negative and positive status. HIV tests are intended to ensure the appearance of HIV antibody in the body, while counseling is provided to determine the level of infection risk from this behavior and how it should behave later after learning the results of HIV tests.

Conclusion

Adolescent knowledge of HIV/AIDS is a fundamental aspect that should be instilled in adolescents as early as possible in efforts to prevent HIV transmission. HIV/AIDS infection can be transmitted through bodily fluids such as semen, sperm, vaginal fluids, blood and breast milk. The incidence of HIV/AIDS in adolescents is most transmitted through contact with bodily fluids when intercourse, namely contamination of semen, sperm and vaginal fluids due to free association and juvenile delinquency. Factors related to HIV/AIDS transmission in adolescents include adolescent gender, family factors, soy environmental factors and technological and communication factors.

Therefore, it is expected that the cooperation of all sectors of both parents, social institutions, and society in general to pay attention to and supervise adolescents from juvenile delinquency and free association to save
the future of adolescents as well as an effort to support
government programs for HIV/AIDS-free Indonesia.

**Conflict of Interest:** The authors have no conflict of
interest with the material presented in this paper.

**Source of Funding:** None.

**Ethical Clearance:** None. This research was
conducted without treating informants

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Molecular & Antifungal Susceptibility Identification of *Candida albicans* Isolated from Samples of GIT Children with Diarrhea in Diyala Province Iraq

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Abstract

Diarrheal diseases in children are a major public health concern in developing countries. Especially species of *Candida*, responsible for causing candidiasis infection (diarrhea), require fast and accurate identification. The correct identification of *Candida* species is of great importance, as it presents prognostic and therapeutic significance, allowing an early and appropriate antifungal therapy. This study was conducted to investigate the incidence of *Candida albicans* associated with diarrhea in children.

This study was included isolates of *Candida* spp. from stool of 100 patients samples with diarrhea and 100 without diarrhea evaluated in AL-Batool Teaching Hospital by phenotypic methods, antifungal susceptibility of *C. albicans* and determination of the *C.albicans* genotypes by Nested PCR, discriminating *C. albicans* from the other *Candida* species. The tests used for phenotypic analysis were culture in SDA and CHROMAgar™ Candida, Phenotypic tests showed green colonies in chromogenic medium. The antifungal susceptibility results of 43 *C.albicans* isolates present that all of *Candida* isolates tested were susceptible to Amphotericin B and fluconazole (100%), while no resistance was observed in *C.albicans* to Caspofungin and Micafungin.

Finally, only one isolate was resistant to flucytosine. Genomic DNAs of all *C. albicans* isolates were amplified by PCR to detect their genotypes using, polymerase chain reaction amplification shows four genotypes (A, B, C, and D). A-genotype showed 4 subtypes and most common subgenotypes (1, 3, 4, and 5).

Kew word: *C.albicans*, Nested PCR, Antifungal susceptibility genotype identification

Introduction

*Candida species* is the genus comprises about 150 yeast species, the genus is composed of a heterogeneous group of organisms, and more than 17 different *Candida* species are known to be etiological agents of human infection; however, more than 90 % of invasive infections are caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* [14].

*Candida* is a part of normal flora of the human body colonizing various anatomical site like oral cavity, digestive tract, vagina and skin[19]. In cases where there is host debilitation, or where there is a change in the local environment promoting *Candida* overgrowth, this led to *candida* infection referred to as candidiasis) [7].

*C albicans* is the most common yeast species isolated from human feces, being identified in 65% of stool samples from healthy adults. Nevertheless, several reports have suggested that it may cause diarrhea. [3].

Diarrhea is a major cause of child morbidity and mortality in socio-economically developing countries. More than one million episodes of diarrhea occur every year among children under five years of age causing approximately 2.5 million deaths [12][16].

Molecular method for direct identification of *Candida* species has proven to be an accurate and rapid method for detection of candidal infections [16][23].

Nested polymerase chain reaction (Nested PCR) is a modification of polymerase chain reaction intended
to reduce non-specific binding in products due to the amplification of unexpected primer binding sites[5].

**Material and Methods**

**Isolation and Identification of Candida spp.**

Stool sample were collected from diarrheal children under 5 years old came to the microbiology laboratory in AL-Batool Teaching Hospital, general stool examination and Stool culturing during two months period (from 1st September 2018 to end November 2018).

All the collected samples were cultured directly, on Sabouraud dextrose agar (SDA) containing Chloramphenicol. Then incubated for 48 hrs. And the only positive samples were cultured on CHROM Agar (CAC).[13]

**Antifungal susceptibility test**

Antifungal susceptibility test was done according to ( Vitek 2 Compact), The YST identification card is based on established biochemical methods and newly developed substrates [2]. Final identification results are presented in approximately 14-18 hours.

The VITEK 2 cards containing serial twofold dilutions of Amphotericin B, Fluconazole, Flucytosine, and Voriconazole,Caspofungin,Micafungin were provided by the manufacture.

**Molecular Methods – based PCR.**

**DNA extraction.**

Genomic DNAs were extracted from *C.albicans* isolates, using a QIAamp DNA Mini Kit according to the manufacturer’s ( Qiagen DNA extraction kit) modified protocol. The purified DNA was stored at 20 ºC until further analysis. Quantus Florometer was used to detect the concentration of extracted DNA in order to detect the purity of samples for downstream applications.

For 1 µl of DNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected, DNA concentration appeared at rate from 2-20 ( ng/µl).

Candida albicans Identification by Nested PCR

In order to identify *Candida albicans* genotypes and subgenotypes on the basis of 25S rDNA, Two sets of primers were used to increase these sensitivity and specificity of the assay. *C.albicans* was classified into five genotypes: A, B, C, D, and E [4],[15].

**Identification of Candida albicans genotypes**

The specific primer pairs used to detect the 25S rRNA were CA-INT-L (ATA AGG GAA GTC GGC AAA ATA CCG TAA) and CINT-R (CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT) [12].

**Identification of Candida albicans subgenotypes**

The primer set ASDcF : 5’-TGATGAACCACATGTGCTACAAG-3’

And pCSCR: 5’-CGCCTCTATTGGTCGAGCAGTAGTC-3’

Is referred to as P-II in this study used for identify subgenotype of *C.albicans* [4].

all primers were synthesized by (Bioneer Co., USA). These primers were suspended in Nuclease-Free water to be 100 pmol/ µl as definitive concentration, as recommended by company protocol, put in freezer until time of use.

**Statistical Analysis.**

All data were statistically analyzed depending on SPSS (Statistical Package for Social Science) version 18 (2009).Chi-square and Yates’s correction was used to compare the variable in this study. Statistical results were considered significant when being under or equal to the 0.05.

**Results and Discussion.**

**Isolation and Identification of Candida spp.**

Morphological culture on SDA, colonies of *C. albicans* on sabouraud dextrose agar were white to creamy, round, soft, and smooth to wrinkled with a characteristic yeast odor as presented in figure 1).[16].
The results of this study are in agreement with [23], in thi-Qar that found the total rate of candida infection was (64.4%) of 264 examined stool specimens at Al-Zahraa health center, Suq Al-Shuyukh district. In another study from Kurdistan, Iraq, 600 stool specimens from children in different age (<2-12 years) were examined not only for Candida spp. but also for enteropathogenic bacteria, parasites, and viruses; Candida spp. could be detected in 1.16% of diarrhea cases [19].

The present study indicate that difference between Candida spp., 43 (64.71%) of the total isolated were identified as C. albicans, 24 (35.82%) isolates were identified as non-albicans, of which Candida tropicalis 8 (11.94%), Candida glabrata 6 (8.95%), Candida krusei 5 (7.46%), Candida parapsilosis 5 (7.46%) as shown in table 1.

<table>
<thead>
<tr>
<th>Candida species</th>
<th>No. of isolates</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>43</td>
<td>64.17%</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>8</td>
<td>11.9%</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>6</td>
<td>8.9%</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>5</td>
<td>7.4%</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>5</td>
<td>7.4%</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>100%</td>
</tr>
</tbody>
</table>

Candida species’ well growth on chromogenic medium in this work supported the fact about this medium has a good performance, less time consuming, and sensitive in detection of Candida species [22]. These results also confirmed that chromogenic agar had a major role in the classification of Candida spp. into C. albicans and non-albicans [3][15].

Antifungal susceptibility test.

In this study, Vitek 2 YST was evaluated using 43 isolates of commonly observed species of Candida (C. albicans). The result generated as the Vitek 2 AST-YSO7 Card evaluated the sensitivity and resistance of Amphotericin B, Fluconazole, Micafungin, Caspofungin, Voriconazole, and Flucytosine.

The antifungal susceptibility results of 43 Candida albicans isolates present that All of Candida isolates tested were susceptible to AMB and fluconazole (100%). The resistance was high in C. albicans against
AMB (34.88%) and then against fluconazole and Voriconazole (32.55% & 25.58%) respectively, while no resistance was observed in *C. albicans* against Caspofungin and Micafungin. Finally, only one isolate of each of the following isolates were resistant to flucytosine in percentage (2.32%). as showed below in (figure.2).

These results were disagreement with (Rehab *et al.*;2011) who found that non of *Candida* isolates were resistant to AMB and only few isolates of *C. albicans* were resistant to FCN

Moreover, This result was agreement with [21] in Diyala who found that the antifungal drugs sensitivity test against *C.albicans* showed that all *C.albicans* isolates (100 %) resistant.

**Molecular Method for Detection of Candida albicans.**

Genomic DNAs of all *C. albicans* isolates were amplified by PCR to detect their genotypes using primer pairs (CA-INT-L and CA-INT-R) by targeting the gene 25S rDNA. polymerase chain reaction amplification shows four genotype(A,B,C,D) as its shown in figure[3].

**Figure 3:** Agarose gel electrophoresis of *C. albicans* genotypes by PCR targeting 25S rDNA on 1% A garose gel, 5 volt/cm for 1.5 hr, stained with ethidium bromide dye. Genomic DNA products generated through The primer pairs CA-INT-L and CA-INT-R, of 450 bp for genotype A in lanes: (26,43,45,50,58, 71,73,76,81,90). 840 bp for genotype B in lanes: (28,65). Both 450 bp and 840 bp for genotype C in lanes: (24,48,49,72,74).1040 bp for genotype D in lanes:(9).
For determination of *C. albicans* on the basis of 25S rDNA, primers CA-INT-L and CA-INT-R that span the site of the transposable intron in the 25S rDNA \(^{15}\) were used in this study. This method has been confirmed as a specific and reproducible method of genotype analysis of *C. albicans* \(^{8}\). PCR targeting 25S rDNA allows *C. albicans* to be grouped into five genotypes A, B, C, D and E\(^{8,10,13,15}\).

In this study, gel electrophoresis profiles defined DNA products as following: 450bp for genotype A, 840bp for genotype B, both 450&840bp for genotype C,1040 pb for genotype D, the study considered this isolate to belong to a new genotype ,None of PCR product in this study were genotype E 1080bp.

In addition to above, the most prevalent *C. albicans* genotype among Gastrointestinal candidiasis isolates on the basis of amplification of 25S rDNA was genotype A (52.6%), followed by genotype C (31.5%), and B (10.5%), and D (5.2%), this result agree with \(^{10,21}\) in Diyala that found the *C. albicans* genotype A is the most frequent genotype in patients but disagree there result that genotype A Followed by genotype B and C respectively.

**Identification of Candida albicans subgenotypes**

*Candida albicans* specific primer pairs (ASDc-F and ASDc-R) were able to successfully amplify the region of the 25S rDNA gene. Polymerase Chain Reaction amplification shows four subgenotypes(1,3,4,5) according product size from any of the genotype A,B,C,D *C.albicans* from 4 isolates ,as it shown in Figure-5.

![Figure 4: Agarose gel electrophoresis of C. albicans subgenotypes by PCR targeting 25S rDNA on 1% Agarose gel, 5 volt/cm for 1.5 hr, stained with ethidium bromide dye. Genomic DNA products generated through The primer pairs ASDc-F and ASDc-R.](image-url)
Another study in Turkey by[15] showed that genotype A (50.2%) was the most predominant genotype among invasive isolates non-invasive isolates from sputum, throat, urine, and feces, followed by genotype C (31.9%), and genotype B (17.9%).

Total of 200 children less five years were included in this study. 67 (67%) children had positive Candidiasis. These highly significant differences between Candida spp., 43 (64.17%) were identified as Candida albicans, 24 (35.82%) isolated were identified as non-albicans.

In this study, we used Nested PCR that can specifically identify C. albicans at the species level, and we found 4 sub genotypes (1, 3, 4 and 5) based on PCR amplification generated by primer pair P-II (ASDc-F and ASDc-R) these genotypes were based on the size of product[10],[11].

Result showed that single amplification product size 526bp as subtype 1 for the C. albicans genotype A,C, and single amplification product size 870bp as subtype 3 for the C. albicans genotype A,B, and amplification product size 1042 as subtype 4 for the C. albicans genotype A,B, and amplification product size 1214 as subtype 5 for the C. albicans genotype D.

Conclusions

From this study we conclude that about 67% of diarrheal cases were associated with Candidiasis.the antifungal test revealed that all of C. albicans isolates were susceptible to AMB and fluconazole while no resistant were observed against Caspofungin and Micafungin . polymerase chain reaction amplification shows four genotype(A,B,C,D).A genotype showed 4 types and most common sub genotypes(1,3,4 and 5).

Funding

I receive no fund to complete this article apart from self-funding.

Conflict of Interest: None to declare.

Ethical Clearance: All the strategies that we had followed were approved by the administration of our hospital and were carried out in accordance with who and the CDC guidelines.

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Markers of Bone Turnover in the Evaluation of Diagnosis and Prognosis of Multiple Myeloma in a Sample of Iraqi Patients

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Abstract

Subjects /This study is aimed to evaluate the bone resorption marker CTX, B-CTX, and bone formation marker Osteocalcin in relation to the clinical presentation as well as to investigate the evidences of the osteoporotic processes by assessing osteoclast bone markers in the different stages of MM patients and comparing these markers- in relation to standard prognostic markers in sample of MM Iraqi patients. – in addition to interpretation of soluble CD138 in relation to prognosis.

Methods/Sixty-five MM (males=41, females=24) patients distributed to different hematology centers in Iraq were enrolled in this cross-sectional study. Their age range was 39-81 years, twelve of them were newly diagnosed and the others were under treatment and distributed all on three stages from the disease according to the international staging system (ISS) : Group A – Stage I (n=21 patients, age mean 57.14±12.25 years), Group B – Stage II (n= 22 patients, age mean of 56.45±11.33 years), and Group C-Stage III (n=22 patients, age mean 60.59±11.55 years). Seven milliliters of venous blood samples were taken from each patient just prior to starting the chemotherapy for the measurement of blood hemoglobin (Hb), serum Creatinine, Calcium, Beat 2 Microglobulin Osteocalcin (OC), Total and Beta C-terminal telopeptide (CTX, BCTX), Parathyroid hormone (PTH), Syndecan-1 (CD138), and both kappa & lambda Free light chain (FLCκ, FLCλ).

Results/Anemia was a general feature of all patients, with a gradual rise (from stage 1 to 3) in the concentrations of creatinine and B2M P=<0.001, calcium P=0.01 and, all other studied markers CTX, BCTX, CD138, FLCκ and FLCλ has significant elevation in comparison among studied groups P=<0.001 with the exception of Osteocalcin ,which showed a general reduction.

Conclusion/Multiple Myeloma patients have increased in bone remodeling throughout the disease course with progressive increment in relation to disease stage --. Plasma cells in MM leads to disturbance in expression and secretion of CD138 in association with disease advancement.

Key words: Multiple Myeloma , bone turnover and C-terminal telopeptide (CTX).

Introduction

Clonal dissemination from malignant plasma cells in the microenvironnement of the bone marrow with the appearance , in blood and urine, of monoclonal protein, are important features of multiple myeloma (MM) which are usually associated with organ dysfunction , extensive lytic bone lesions and osteoporosis (¹). Patients with MM usually complain of fatigue , bone pain , easy bruisability, bleeding and recurrent infections which are manifested by hypercalcemia, lytic bone lesions, hyperviscosity, thrombocytopenia, hypo-gammaglobulinemia and many others (²,3) .

The myeloma cells are synergized by bone microenvironment which include fibroblasts, osteoblasts and osteoclasts(⁴). Increased rate of bone resorption and remodeling with a morphological assessment of plasma cells are important criteria for the evaluation of symptomatic myeloma (⁵). Precipitation of light chains
casts within the distal and collecting tubules-as a result of increased production of M-protein could be a cause of development of what is called myeloma kidney together with hypercalcemia, free light chain proteinuria, lack of hydration, hyperuricemia, and nephrotoxic medications and consequent recurrent bacterial infections (6,7).

Hypercalcemia can hasten and disturb renal insufficiency (8).

Of the prognostic markers β2 microglobulin its raised concentrations has been consistently reported in conditions characterized by lymphocyte activation and (or) proliferation. It is eliminated via the kidneys (9).

Circulating serum free light chain immunoglobulins are among the prognostic factors. These comprise two types: Kappa (K) and Lambda (λ). The ratio between the two types is increased in plasma cell tumors (10).

Plasma C-reactive protein (CRP) may rise rapidly after an acute inflammatory stimulus, mostly due to increased synthesis by the liver (11), and can serve as an important indicator from survival and post-treatment follow-up in cancer patients (12). Other recent prognostic criteria could be considered for MM as syndican-1 (CD138), osteocalcin, and Beta C-Terminal Telopeptide (BCTX) are still debated.

**Subjects and Methods**

Hospital-Based cross-sectional research was conducted over eleven months from May 2018 to June 2019. A total of 65 Multiple Myeloma (MM) patients participated in the study who were subjected to physical examination and diagnosed by hematologists with multiple myeloma from both genders (based on the Diagnostic criteria of the International Myeloma Working Group - IMWG). They were distributed to different Departments of hematology from Al-Imamain Al-Kadhimain Medical City, Al-Yarmuk Hospital, Baghdad Medical City teaching laboratories, Mirjan teaching Hospital in Hilla, and center of hematology and oncology in Basra.

The age range was 39 – 81 years. A group (12) of newly diagnosed patients emerged. The 65 patients were grouped into 3 stages according to the international staging system (ISS): Group A – Stage I (n=21 patients, age mean 57.14±12.25 years), Group B – Stage II (n=22 patients, age mean of 56.45±11.33 years), and Group C-Stage III (n=22 patients, age mean 60.59±11.55 years).

Full data were collected from each patient using a preformed questionnaire. Initial laboratory results were recorded from tests performed for the patients. Serum urea, creatinine, and calcium were determined by spectrophotometric methods. Moreover, a complete blood count and ESR, serum Immunofixation Electrophoresis, Imaging bone surveys, bone marrow aspirate, and bone marrow biopsy results were registered for each patient.

Patients excluded from the study were those who had Liver disease, active infections (human immunodeficiency virus, HIV, Hepatitis B, or C), and pregnant or breast-feeding women. For each patient the following laboratory tests were done: Free light chains test Kappa (FLC-k) & Lambda (FLC-l), B2Microglobulin (B2-MG), Osteocalcin (OC), C-terminal telopeptide (CTX), Beta C-terminal telopeptide (BCTX), Parathyroid hormone (PTH) and Syndecan-1 (CD138). All tests were done by the ELISA technique.

The Practical part was performed in the Department of Chemistry and Biochemistry, College of Medicine / Al Naharin University Research Laboratories. Test kits were purchased from Cusabio-China for PTH, CD 138, and B2MG, and from Melsin-China for OC & CTX, and from the Bio Vender-Czech Republic for free light chain (Kappa & Lambda). The study approved by IRB at College of Medicine/Al Naharin University.

**Results**

The most prevalent presenting features were anemia (43.08%) followed by back pain (29.23%) and renal complications (13.85%) as in table 1.

A gradual increase in serum B2MG, calcium and creatinine with plasma cells is present from stages I to III (P<0.001), but no marked differences in Hb % and immunofixation results (table-1).

For FLCk, and κ/λ ratio, stage I and II showed a comparable level of these markers with no significant differences, but both stages had significantly lower levels than stage III, (P=<0.001). as in table.
Serum parathyroid hormone, CD138 and total FLC, show comparable levels between stage II and stage III, while both stages were significantly higher than stage I (P<0.001).

There was a gradual increase in the β-CTX marker from stage I to stage III. (P<0.001). as in table

There is a gradual reduction in serum osteocalcin from stage 1 to III (P< 0.001).

### Table 1: Association of common laboratory findings with disease staging

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stage I (n=21)</th>
<th>Stage II (n=22)</th>
<th>Stage III (n=22)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>10.15±0.34</td>
<td>10.08±1.17</td>
<td>9.86±0.63</td>
<td>0.453</td>
</tr>
<tr>
<td>Plasma cell%</td>
<td>24.95±10.84a</td>
<td>37.09±10.45b</td>
<td>51.59±9.29c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.942±0.33a</td>
<td>2.04±0.53b</td>
<td>2.91±0.59c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>10.11±0.87a</td>
<td>10.51±1.46a</td>
<td>11.35±1.53b</td>
<td>0.01</td>
</tr>
<tr>
<td>β2-M (mg/L)</td>
<td>3.57±0.95a</td>
<td>5.92±0.29b</td>
<td>8.34±0.77c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Immuno-Fix.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG-K</td>
<td>12(57.14%)</td>
<td>7(31.82%)</td>
<td>15(68.18%)</td>
<td>0.165</td>
</tr>
<tr>
<td>IgG-L</td>
<td>5(23.81%)</td>
<td>9(40.91%)</td>
<td>2(9.09%)</td>
<td></td>
</tr>
<tr>
<td>IgA-K</td>
<td>3(14.29%)</td>
<td>3(13.46%)</td>
<td>4(18.18%)</td>
<td></td>
</tr>
<tr>
<td>IgA-L</td>
<td>1(4.76%)</td>
<td>3(13.46%)</td>
<td>1(4.55%)</td>
<td></td>
</tr>
</tbody>
</table>

* a, b and c different small letters indicate significant differences

### Table 2: Association of specific laboratory investigation with disease staging

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stage I (n=21)</th>
<th>Stage II (n=22)</th>
<th>Stage III (n=22)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (pg/ml)</td>
<td>137.14±25.87a</td>
<td>160.54±28.49b</td>
<td>165.95±26.25b</td>
<td>0.002</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>12.43±5.78a</td>
<td>10.60±6.35a</td>
<td>6.22±4.08b</td>
<td>0.001</td>
</tr>
<tr>
<td>CTX (ng/ml)</td>
<td>18.24±5.80a</td>
<td>24.08±5.02ab</td>
<td>29.65±6.99b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BCTX (ng/ml)</td>
<td>5074.3±1742a</td>
<td>6824.5±1507b</td>
<td>8497.7±2099c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD138 (ng/ml)</td>
<td>246.8±115.5a</td>
<td>443.6±183.8b</td>
<td>495.6±160.5b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FLCκ (ng/ml)</td>
<td>15.19±9.83a</td>
<td>15.60±14.51a</td>
<td>53.35±25.31b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FLCλ (ng/ml)</td>
<td>17.19±10.60a</td>
<td>51.35±39.30b</td>
<td>22.10±24.69a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>κ/λ ratio</td>
<td>1.33±0.99a</td>
<td>1.02±1.266a</td>
<td>4.95±6.62b</td>
<td>0.002</td>
</tr>
<tr>
<td>Total FLC (ng/ml)</td>
<td>32.30±8.98a</td>
<td>66.96±32.12b</td>
<td>75.45±26.38b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* a, b, c different small letters indicate significant differences
The Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value for the detection of new MM cases. The patients were divided into two groups regardless of staging: newly diagnosed (12 cases) and longstanding (53 cases) the results revealed:

For osteocalcin, the area under the curve (AUC) was 0.553 (95%CI= 0.35-0.756), p= 0.565. The sensitivity and specificity of the test at osteocalcin cut off = 7.4 mg/dl were 0.604 and 0.587 respectively.

For CD138 the AUC was 0.748, 95%CI= 0.629-0.891, p= 0.008. The sensitivity and specificity of the test at CD138 cut off= 330 ng/ml was 0.68 and 0.677 respectively.

better results were obtained from total FLC, FLCκ or FLCλ:

For FLCκ, the AUC= 0.809, 95%CI= 0.64-0.971, p=0.001. The sensitivity and specificity of the test at FLCκ cut off= 9.5 ng/ml was 1.0 and 0.677 respectively.

For FLCλ, the AUC= 0.816, 95%CI= 0.7-0.932, p=0.001. The sensitivity and specificity of the test at FLCλ cut off= 8.3 ng/ml was 0.717 and 0.677 respectively.

while total FLC was the best; the AUC was 1.0, 95%= 1.0-1.0, p<0.001. The sensitivity and specificity of the test at total FLC cut off= 28.35 ng/ml were 1.0 for both.

Beta-2-microglobulin was found to be significantly decreased in longstanding patients compared with the newly diagnosed, and hence it was analyzed with a separated ROC curve. The AUC was 0.601, 95%CI= 0.45-0.751, p=0.279. The sensitivity and specificity of the test at β2-microglobulin cut off= 5.55 mg/L were 0.883 and 0.66 respectively.

Fig. 1: Receiver Operating Curve for Some Selected Markers in The Context of Discrimination Between Newly Diagnosed and Longstanding Multiple Myeloma
Fig. 2: Receiver Operating Curve for B2-Microglobulin in the Context of Discrimination Between Newly Diagnosed and Longstanding Multiple Myeloma.

Fig. 3: Receiver Operating Curve for Total Free Light Chains in the Context of Discrimination Between Newly Diagnosed & Longstanding Multiple Myeloma.
Discussion

The distribution of MM patients among the three stages of the disease is more or less, the same which makes the comparision more convenient.

The hypercalcemia shown by the present patients could be attributed to the raise in the osteoclast-activating factor cytokines, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) is a member of the TNF ligand/receptor superfamily \((13)\).

It is a major mediator of osteoclast differentiation and activation and is over-expressed in the microenvironment from myeloma marrow. It is antagonized by osteoprotegerin (OPG), which is downregulated in myeloma lesions. Thus, there is a net increase in RANKL and a decrease in OPG in multiple myeloma, leading to osteoclast hyperactivity \((14)\).

Hypercalcemia associates PTH which is reported to increase in MM \((15)\).

The increase in B2 Microglobulin (B2MG) is a common feature of MM which was attributed to a mutation in the B2MG gene which has been shown to result in hypercatabolic hypoproteinemia. Diseases associated with B2M include immunodeficiency 43 and amyloidosis. High serum B2MG values can be thought of as a result of increased “synthesis” whether owing to increased expression, increased HLA turnover, cell proliferation, cell lysis, or some combination of these and not of decreased renal clearance \((16,17)\).

High levels of B2MG are associated with greater tumor burden and renal failure. This is manifested in this study by the high concentration of serum creatinine which was seen to be more prominent in 25% of myeloma patients \((18)\).

Pathologic injury of myeloma kidney is the presence of monoclonal light chains in the tubules in the type of thick, regularly overlaid, cylindrical throws. These casts contain - Tamm-Horsfall protein \((19)\). Uromodulin, is a protein unique to the kidneys. It coats the epithelium luminal side and is the most abundant in human urine \((20)\).

Osteocalcin is expressed directly by Osteoblasts. Most historical studies have focused on characterizing OC as a fracture indicator and only as a part of bone metabolism \((21)\). Normally bone homeostasis depends on the coupling of OCs and Osteoblasts. This coupling mechanism is lost in MM leading to an expansion of osteoclastic action with consequent bone resorption and diminished arrangement \((22,23)\).

The increase in CTX and Beta-CTX could be due to the secretion of a mixture of acid and neutral proteases by osteoclasts during bone resorption. This had made them specific markers for the degradation of type1 collagen \((24,25)\). This study shows an increase in the CTX and BCTX levels according to MM stages, the highest being in the third stage. These findings agree with Schiano C, Soricelli A. 2019, In vitro evidence supports a role for CD138 (syndecan-1) in the adherence of plasma cells to the stromal matrix of the bone marrow via an association with type I collagen.

From studies on monoclonal antibodies human syndecan-1 seems to be plasma cell-specific among hematopoietic elements and appears to be involved in the process of carcinogenesis \((26)\).

In people with osteoporosis, bone turnover markers may be useful for evaluating the response to anabolic and antiresorptive therapies, for evaluating therapy compliance or for suggesting potential secondary osteoporosis. A great deal remains to be learned about how bone turnover markers can be used to track the impact of stopping bisphosphonate therapy (e.g., determining a threshold above which restart therapy should be taken). several studies are needed to research the use of bone turnover markers to evaluate the bone safety of new drugs \((27)\).

The ROC test, in this study, shows clearly the differences in the specificity and sensitivity of the different bone biomarkers in MM, the net result of this test reveals that the Total free light chain remained the best by getting sensitivity and specificity of 1 for each.

At last we may say that careful monitoring of Bone markers levels at regular intervals is recommended with the emphasis on the control of parathyroid hormone and reduction of serum levels of bone markers by any possible mean.
Therefore, the bone remodeling markers are pivotal in evaluation of progression of MM patient which indeed may attribute in treatment decision as well as the monitoring of treatment results according to specific therapy.

**Conclusion**

Multiple Myeloma patients have increased in bone remodeling throughout the disease course with progressive increment in relation to disease stage. Plasma cells in MM leads to disturbance in expression and secretion of CD138 in association with disease advancement.

-The ethical clearance of my article taken from the Institutional Ethics Committee in Medical College / fallujah University / Iraq ,this ethical clearance Installed and written in materials and methods of my article.

**The Source of funding**: Self).

**Conflicts of Interest**: Not found

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Histological Effect of Androgenic Anabolic Steroids on Liver

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Abstract: Anabolic androgenic steroids (AAS) they are the synthetic type of the natural male sex hormone (testosterone), they are widespread used amongst athletes to enhance performance. Abuse of AAS is common amongst players and it is usually accompanied by a many medically based complications. It was reported that hepatic problems include cholestasis, elevation of liver enzymes e.g. aminotransferases, as well as, benign hepatic adenomas, jaundice and to less extent of hepatocellular carcinoma was associated with their use.

A total of (12) adult New Zealand rabbits (Oryctolagus cuniculus) male, aged one year was selected. 2 control animals and 10 were treated with injections intramuscularly of the AAS, nandrolone decanoate (15 mg/kg) three times a week for 12 weeks. At the end of experiment the animals were sacrificed and the liver was dissected out and fixed using 10% buffered formalin-saline to prepare blocks for staining with haematoxylin and eosin for histological examination using the light microscope.

The liver of treated animals reveals that there are a mild to severe vascular congestion. There is swelling of hepatocytes, and inflammatory cell infiltration. Perivascular fibrosis, cellular necrosis was observed in other slides with sinusoidal congestions and extrabiliary bile pigments deposits.

The benefit of AAS comes with unwanted side effects. The total volume of the hepatocytes and sinusoids were increased in the studied animals. The total number of hepatocytes nuclei in experimental group also increased. The damage of the liver cells or at least increased permeability of the hepatocellular membrane which is noticed in the present work could explain the increment of plasma levels of liver enzymes which was studied by other researchers, as they notice an elevation liver enzymes activity such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT), and lactate dehydrogenase (LDH).

Patients and physicians must kept in mind that the sequelae of AAS abuse are life threatening. So people should be aware about the complications of AAS use and a periodic examination of liver function and check out should be done to those treated groups.

Keywords: Androgenic anabolic steroid, histology, liver, liver enzymes.

Introduction

Anabolic androgenic steroids they are the synthetic type of the natural male sex hormone (testosterone), have been in commonly use among athletes to improve performance for many years. The hepatotoxicity risk is come to light as the dramatic increase in the use of AAS by athletes and young people. With the widespread use of AAS has come an associated increment of alertness of the adverse effects and sometimes serious sequelae of anabolic androgenic steroids use. Nonetheless, there seems to be a constant increase in the use of AAS by players and for aesthetic functions. Recent evaluations put AAS use in the Sweden and USA at 1% of the population and it can rationally accepted that the amounts of usage in Canada are parallel1. The online shopping using internet has elevates the illegal obtainability of these drugs without medical prescription and the buyers are usually unconscious of the dangers of administrating these drugs. AAS abuse is common amongst sportsmen and is accompanied by a lot of medical problems.. documented

1 DOI Number: 10.37506/ijfmt.v15i1.13621
hepatic disease including cholestasis, benign hepatic adenomas, increment of aminotransferases, jaundice and in rare cases the development of hepatocellular carcinoma. High doses of AAS may lead to temporary sterility, and testicular atrophy. There are 3 common dosage forms in which AAS are administered: oral pills, skin patches and injectable steroids. Oral dosage forms are the most suitable. AAS administered by oral intake is rapidly absorbed, but it is converted largely to inactive metabolites, and only about one-sixth is existing in the active form. AAS can be administered by parenteral route, but it has irregular extended absorption time and greater activity in cypionate ester, enanethate, proionate or undecanoate form. Parenteral steroids are typically administered intramuscularly, not intravenously; to avoid rapid changes in the concentration of the drug in the blood stream. Ciba was introduced Methandrostenolone (Dianabol) as an AAS by in the 1960s. Methandrostenolone was one of the AAS used to improve athletic act by the former East German Olympic programm. many adverse effects of AAS which include acne, gynecomastia, mood changes (aggressiveness) and testicular atrophy were recorded to accompany the use of this AAS. Stanozolol, another carbon-17- alkylated AAS, has been reported previously to cause acute renal failure and severe cholestasis in a young athlete. The use of AAS and vitamin supplementation may associated with acute renal injury as it was reported in two male athletes. Most of the synthetic androgens and anabolic agents are 17-alkyl-substituted steroids. Hepatic dysfunction is often associated with the administration of these drugs, e.g. increase in sulfobromophthalein retention and aspartate aminotransferase (AST) levels. Alkaline phosphatase values are also elevated. These changes occur early usually in the period of treatment, and the amount is proportionate to the dose. Bilirubin levels sometimes elevated until clinical jaundice is apparent. Cholestasis of the intrahepatic bile ducts has been reported to be caused by Methandrostenolone resulting in hyperbilirubinemia elevated aminotransferases, and clinical jaundice. nandrolone decanoate has androgenic: anabolic activity 1:2.5-1:4 compared to testosterone.

Materials and Methods

A total of (12) adult New Zealand rabbits (Oryctolagus cuniculus) male, aged one year with body weight of (1.7-2.2) Kg was selected for the study. Animal were kept in steel cages with grid floor and fed by fresh trefoil diet and water supply ad libitum. They kept at room temperature (25 -27°C) and 12-hr light -dark cycles with good ventilation. Two animals served as control group. The remain 10 were the treated group which received injections of the anabolic-androgenic steroid, nandrolone decanoate (15 mg/kg) intramuscularly three times a week for 12 weeks. At the end of the experiment, the animals were sacrificed by exsanguinations after anesthesia using an intramuscular injection of ketamine (3.5 mg/ kg) and xylazine (5 mg/ kg). The liver was taken and dissected out and fixed using 10% buffered formalin-saline to prepare blocks for staining with haematoxylin and eosin for histological examination using the light microscope.

Results

For the study of the general histology of the liver, haematoxylin and eosin stains were used. The architecture of the liver slides of control group of animals, shows that the liver consist of hepatic lobule, each lobule consists of hepatocytes arranged in rows radiated from the central vein. These hepatocytes are separated by hepatic sinusoids, between adjacent lobules there is the portal area (or triad) a branch ofhepatic artery , branch of portal vein, and bile duct are the content of this triad. On examination of liver of treated animals; it reveals that there are a mild to moderate and some times sever vascular congestion. There is swelling of hepatocytes, and inflammatory cell infiltration. There is swelling of hepatocytes, and inflammatory cell infiltration (figure 2). Perivascular fibrosis, cellular necrosis was observed in other slides with sinusoidal congestions and extrabiliary bile pigments deposits (figure 3 and 4 a &b).
Figure 1: histological appearance of normal liver. See the central vein, the arrangement of hepatocytes and sinusoids. H&E stain. X400.

Figure 2: moderate to severe vascular congestion of the liver (arrow) with inflammatory cell infiltration (arrow head). H&E stain. X400.
Figure 3: Perivascular fibrosis and cellular necrosis of hepatocytes. H&E stain. X400
B

Figure.4 A: moderate vascular congestion of the liver with inflammatory cell infiltration (arrow).
Fibrosis and bile pigment extravasation (arrow head).

B: severe vascular congestion with cellular necrosis. H&E stain. X400

Discussion

The advantages of anabolic steroids, unfortunately associated with unwanted side effects. Many organs demonstrate short and/or prolong side effects, and the use of AAS are accompanied by changes in the liver. The use of Anabolic androgenic steroid is not only by athletes who looking for better performance\textsuperscript{16}, weightlifters and bodybuilders, as well as prisoners are also show a high misuse levels \textsuperscript{17}. The availability of the AAS on the internet marketing and public gyms facilitate the ability of a person to get it in illegal ways, without medical prescription. As a consequence, the patients are able to take AAS without informing the physicians and in the same time, the doctors are less likely to be aware of the possibility of the use of AAS the non athlete population \textsuperscript{3}.

The present study clearly showed the effect of anabolic steroid use/or misuse on the liver histology. The histological changes on the liver which has been considered in the present work range from mild to severe vascular congestion with infiltration of chronic inflammatory cells, liver sinuses were congested, and collections of glycogen fibers and fatty degeneration appeared, some slides showed bile plugs in hepatocytes and canaliculi and also outside the cells and canaliculi. The effect of anabolic steroid had been studied previously on kidneys, pancreas from histological point of view. The side effect of AAS on the liver also studied
thoroughly but mainly concentrated on alterations on liver enzymes and the possibility of developing hepatic adenoma and carcinoma. The hepatic cellular damage caused by anabolic steroid occur also on ultrastructural level as shown by other studies, where the mitochondria showed increase in size and the appearance of crystalline inclusions, also there is increase in cytoplasmic volume. Most of the synthetic androgens and anabolic agents are 17-alkylsubstituted steroids. It is widely known that two chief mechanisms of liver injury induced by drugs;they are intrinsic and the idiosyncratic hepatotoxicity. Hepatocellular damage occurs by Intrinsic hepatotoxins in a way depending on the dose either in a direct way by the drug or indirectly by its metabolite. Some of the drugs, like acetaminophen, cause intrinsic hepatotoxicity, but most of this category agents are due to industrial toxins, household, or environmental toxins. The drugs that lead to idiosyncratic liver injury could be classified into metabolic and immunological categories.

In the first category, the drugs is metabolized into metabolite which is toxic in susceptible persons, while in the later is similar to “drug allergy” or sensitivity following predisposition to the drugs. Hepatocellular necrosis is the manifestation of intrinsic hepatotoxicity with little amount of inflammation. Meanwhile idiosyncratic drug responses usually appears with inflammation-dominant liver injury. These facts explain the damage occurred to hepatocytes and the changes in liver structure of animals treated with anabolic steroids which were observed in previous studies and the present work. The mechanism of action of steroids on the cells, can be summarized by that as they are tetracyclic cyclopenta[a]phenanthrene formed compounds which are able to pass across cell membranes and to bind to the cytoplasmic receptors, to form a new complex which binds to the DNA. After it bind to DNA, the steroid-receptor complex will starts a sequelae of process that finally leads to the making of cellular structures and proteins. positive nitrogen balance is the final result for cells. Inspite of the wide range of reactions caused by the different androgenic anabolic steroids compounds, all androgenic anabolic steroids bind directly to one androgen receptor (AR).

AAS were stimulate the synthesis of DNA in a potency between weak to strong effect and alteration to A-ring structure in a combination with non-polar substitution at 17alpha-positioned looks to be necessary for their activity. Previous studies by Dousta and Noorafshan revealed that liver volume and weight in mice treated with anabolic steroids increased in comparison with that of the control group. the hepatocytes and sinusoids showed increment in their volume in the treated animals, too. The total number of hepatocytes nuclei in experimental group increased by 20%. These findings are consistent with the results in this study as there were congestion of sinusoids and increase of hepatocytes size. The damage of the liver cells which is seen in the present work could explain the increment of plasma levels of liver enzymes which is studied to by other researchers, as they notice an elevation in plasma activity of enzymes of the liver e.g. aspartate aminotransferase (AST), alkaline phosphatase (AP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and gamma glutamyl transpeptidase (GGT). These enzymes are found in hepatocytes in high level an increase in the plasma levels of these enzymes is a mark which reflect damage of liver cells or increased permeability of the hepatocellular membrane, at least. The anabolic steroid, as consequence to their effect on the liver, could also affect free fatty acids metabolism by the liver and lead to the accumulation of triglycerides within the hepatocytes. This was appeared in the present work as fatty degeneration of fatty changes of the liver which noticed in some slides, which called steatosis, and it is consistent with other studies that observed such hepatic changes due to anabolic steroids use.

The high doses of alkylated androgens can produce peliosis hepatica, cholestasis, and liver failure. They lower the level of plasma HDL2 and may elevate LDL. Hepatic carcinomas and adenomas also have been reported. Gurakar et al. and another researchers studies showed the hepatotoxic effect of anabolic steroids especially in supraphysiologic doses, as that taken by bodybuild. Some studies reported development of hepatic tumor due to the use of anabolic steroids; this is confirmed by the regression of tumor size and return of liver function to normal by the cessation of steroid usage. Our study did not prolonged enough to elicit such changes in the studied animals. Ramachandran and Kakar study on the liver of patient use anabolic anabolic steroids didn’t show inflammation nor hepatocellular injury, their findings were inconsistent with the results of the present work. We agree with them in part in the occurrence of cholestasis in the anabolic steroids treated livers. Finally, Patients should be noted...
that the sequelae of AAS abuse are life threatening. Meanwhile, the athletes and bodybuilders are aware of the legal and social ramifications of steroid abuse, they must reminded about its medical risks.

**Ethical Clearance:** The animal were sacrificed under the ethical committee approval of college of medicine, university of Al-Qadisiyah.

**Source of Funding:**

The research is made entirely by self – funding.

**Conflict of Interest:** The authors declare that there is no conflict of interest with others.

**References**


Teachers’ Attitude Toward Children with Attention-Deficit Hyperactivity Disorder (ADHD) at Primary Schools in Al-Nasiriyah City, Iraq

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¹Ministry of Health, Al- Nasiriyah , Iraq. ²Professor, University of Karbala, College of Nursing, Karbala, Iraq

Abstract
The aim of the current study is to identify teachers’ attitude about attention-deficit hyperactivity disorder (ADHD) and its relation to their demographic characteristics. A descriptive study design was carried out among 250 teachers at primary schools in Nasiriyah city was selected by using convenience sample type. The data is collected by using Google classroom through distribution of the attitudes questionnaire to primary school teachers which composed of 22 items. The highest percentage 36.8% of teachers at age 41-50 years old, 52.8% were females, 70.8% were urban residence, 54.5% were married, 53.6% had barely sufficient income, 62.8% had bachelor graduated, 56.4% teach the classes (4-6) and 48.8% teach in male school. The majority 95.6% of teachers had neutral attitude toward ADHD and the grand mean was 1.93. There is significant statistical difference in teachers’ attitude with regard to their gender. Majority of teachers had neutral attitude About ADHD among children. Teachers attitudes is affected by gender. It is important to teach teachers about how to deal with child who had ADHD. Assign work that suits the student’s skill level, and give appropriate supervision to ADHD students.

Keywords: Teachers, Attitude, children, attention-deficit hyperactivity disorder (ADHD)

Introduction
Attention Deficit Hyperactivity Disorder (ADHD) is the most commonly studied and diagnosed psychiatric disorder in children, affecting 3 to 5% of children globally. Childhood ADHD can be a disabling illness. If severe forms are left untreated it interferes significantly with the child’s education, interpersonal relationships and ability to maintain a generally positive sense of self. Ill effects of the illness go beyond the child, having detrimental effect on family relationships. ADHD is associated with significant psychiatric co-morbidity, 50-60% of affected children meeting criteria for at least one additional psychiatric disorder. Thirty to 50% of those diagnosed with ADHD in childhood continue to have symptoms into adulthood.¹,²

Attention Deficit Hyperactivity Disorder is one of the most common psychiatric disorders among children and adolescents; the symptoms are hyperactivity, attention deficit and impulsiveness. ADHD co-occurs with some other disorders and the patients go through many problems at home, school and social environments. Based on a literature review, the prevalence of ADHD was 6.8% among children and adolescents. The overall prevalence is 9.7% among elementary school students of Tabriz, North-west of Iran, with a higher rate among the children of illiterate parents.³

The public stigmatizes children with symptoms of ADHD more than children without ADHD symptoms, adding a diagnostic label is associated with only marginally higher rates of stigma.⁴

Teachers play an additional role as gatekeepers to treatment and may elect to accept or deny access to evidence-based treatments for children. teachers not only struggle in the decision to consent to a medication trial, but often revisit their decision. Teachers report
fears of the long-term negative effects of stimulants, even if their child has a positive response to the medication. Furthermore, teachers report trial-stopping their children’s medication without approval from the prescribing doctor. Clearly, some teachers are ambivalent about employing an evidence-based intervention for their children with ADHD, due to their concerns.\(^{(5)}\)

Teachers attitudes are also important due to their effect on actual treatment-seeking behaviors and subsequent treatment adherence. Positive teacher’s attitudes towards the benefits of stimulant medications for ADHD predict later adherence to medication and perceived medication acceptability. Teachers are less willing to engage in both psychosocial and pharmacological treatments for their children if they do not feel knowledgeable about or endorse the acceptability of these treatments. Unsurprisingly, willingness to engage in treatment for children is positively related to teacher’s views on the acceptability and helpfulness of treatment. These results further support the importance of teacher’s knowledge in predicting treatment-seeking and adherence behaviors\(^{(6,7)}\).

Knowledge and attitudes may impact several important outcomes. For example, it has been suggested that gatekeepers who lack knowledge about ADHD may overlook behaviors signifying a child in need of assistance, and they may provide unreliable information to medical practitioners about the effects of medication. Similarly, it has been suggested that gatekeepers’ attitudes about ADHD may influence their selection of a teaching approach, their willingness to implement interventions, their chosen behavioral management strategies, and classmates’ perception of the child with ADHD\(^{(8)}\).

Teachers play a key role in many aspects of ADHD treatment planning and implementation. Insufficient knowledge and negative attitudes towards ADHD and its treatment among them result in a lack or improper implementation of management recommendations leading to treatment failure. Another explanation for this problem is the teachers’ knowledge about this disorder and their attitude toward treatment, as it has been seen that those teachers who had more knowledge about the disorder used pharmacologic and non-pharmacologic therapies more than the others\(^{(9)}\).

**Methodology**

Descriptive study design carried out among 250 teachers at primary schools in Nasiriyah city, they are selected by using non probability sampling (convenience sample). The study instrument is a questionnaire designed according to the study purpose. The study instrument composed of 2 parts. 1st Teacher’s Demographic Characteristics, 2nd attitude scale composed of 22 items. The attitude questionnaire had been scored and rated on three levels Likert scale, (3) points for agree, (2) points for not certain answer and (1) point for the disagree which assessed by cutoff point (0.66) due to scores (1, 2 and 3) respectively. Scores of responses are categorized according to the following level of attitudes: (1-1.66) = negative level of attitudes, (1.67-2.33) = neutral level of attitudes and (2.34-3.00) = positive level of attitudes. There are some items in reverse sentences in attitude questionnaire, in this case the score will be reversed (1) points for agree, (2) points for not certain answer and (3) point for the disagree. This items are (1,2,5,6,7,8, and 11).

The instrument face validity was determining through a panel of experts. The reliability of the questionnaire determined by Cronbach’s alpha for the internal consistency reliability. The data is collected by using google classroom to communicated with the teachers and gathering information. The data is analyzed by using descriptive and inferential statistical analysis through using (SPSS).
## Results

Table 1: Distribution of the sample according to their socio-demographic characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>32</td>
<td>12.8</td>
</tr>
<tr>
<td>31-40</td>
<td>89</td>
<td>35.6</td>
</tr>
<tr>
<td>41-50</td>
<td>92</td>
<td>36.8</td>
</tr>
<tr>
<td>51-60</td>
<td>37</td>
<td>14.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>132</td>
<td>52.8</td>
</tr>
<tr>
<td>Male</td>
<td>118</td>
<td>47.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>73</td>
<td>29.2</td>
</tr>
<tr>
<td>Urban</td>
<td>177</td>
<td>70.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>81</td>
<td>32.4</td>
</tr>
<tr>
<td>Married</td>
<td>136</td>
<td>54.4</td>
</tr>
<tr>
<td>Widowed</td>
<td>20</td>
<td>8.0</td>
</tr>
<tr>
<td>Divorced</td>
<td>6</td>
<td>2.4</td>
</tr>
<tr>
<td>Separated</td>
<td>7</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Monthly income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>24</td>
<td>9.6</td>
</tr>
<tr>
<td>Barely sufficient</td>
<td>134</td>
<td>53.6</td>
</tr>
<tr>
<td>Sufficient</td>
<td>92</td>
<td>36.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>89</td>
<td>35.6</td>
</tr>
<tr>
<td>Bachelor</td>
<td>157</td>
<td>62.8</td>
</tr>
<tr>
<td>Master and above</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Class in teaching</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1-3</td>
<td>67</td>
<td>26.8</td>
</tr>
<tr>
<td>Class 4-6</td>
<td>141</td>
<td>56.4</td>
</tr>
<tr>
<td>Both</td>
<td>42</td>
<td>16.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>School type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>83</td>
<td>33.2</td>
</tr>
<tr>
<td>Male</td>
<td>122</td>
<td>48.8</td>
</tr>
<tr>
<td>Mixed</td>
<td>45</td>
<td>18.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>250</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The result in table (1) shows that the highest percentage (36.8%) of teachers at age (41-50) years old, (52.8%) of them were females, (70.8%) of them were urban residence, (54.5%) were married, (53.6%) had barely sufficient income, (62.8%) had bachelor graduated, (56.4%) teach the class (4-6) and (48.8%) teach in male school.
Table 2: The nature of teachers’ attitude about ADHD

<table>
<thead>
<tr>
<th>Items</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
<th>Mean</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-ADHD is a behavioral disorder that should not be treated with medication. R</td>
<td>186</td>
<td>17</td>
<td>47</td>
<td>1.44</td>
<td>–ve</td>
</tr>
<tr>
<td>2-Children who cannot sit still in class simply need to be disciplined or punished. R</td>
<td>44</td>
<td>8</td>
<td>198</td>
<td>2.61</td>
<td>+ve</td>
</tr>
<tr>
<td>3-I would feel frustrated having to teach an ADHD. R</td>
<td>189</td>
<td>5</td>
<td>56</td>
<td>1.46</td>
<td>–ve</td>
</tr>
<tr>
<td>4-ADHD is a legitimate educational problem. R</td>
<td>83</td>
<td>5</td>
<td>162</td>
<td>2.31</td>
<td>N</td>
</tr>
<tr>
<td>5-Having an ADHD child in my class would disrupt my teaching. R</td>
<td>188</td>
<td>4</td>
<td>58</td>
<td>1.48</td>
<td>–ve</td>
</tr>
<tr>
<td>6-DHD children should be taught by special education teachers. R</td>
<td>137</td>
<td>4</td>
<td>109</td>
<td>1.88</td>
<td>N</td>
</tr>
<tr>
<td>7-Most students with ADHD do not really disrupt classes that much.</td>
<td>156</td>
<td>1</td>
<td>93</td>
<td>2.25</td>
<td>N</td>
</tr>
<tr>
<td>8-Children with ADHD should be taught in the regular school system.</td>
<td>116</td>
<td>5</td>
<td>129</td>
<td>1.94</td>
<td>N</td>
</tr>
<tr>
<td>9-The extra time teachers spend with ADHD students is at the expense of students without ADHD. R</td>
<td>180</td>
<td>1</td>
<td>69</td>
<td>1.55</td>
<td>–ve</td>
</tr>
<tr>
<td>10-Other students do not learn as well as they should when there is an ADHD child in the class. R</td>
<td>136</td>
<td>5</td>
<td>109</td>
<td>1.89</td>
<td>N</td>
</tr>
<tr>
<td>11-ADHD children misbehave because they are naughty. R</td>
<td>177</td>
<td>2</td>
<td>71</td>
<td>1.57</td>
<td>–ve</td>
</tr>
<tr>
<td>12-ADHD children cannot change the way they behave. R</td>
<td>109</td>
<td>9</td>
<td>132</td>
<td>2.09</td>
<td>N</td>
</tr>
<tr>
<td>13-ADHD children misbehave because they do not like following rules. R</td>
<td>186</td>
<td>8</td>
<td>56</td>
<td>1.48</td>
<td>–ve</td>
</tr>
<tr>
<td>14-Combination of medication and behavior management is best for treating ADHD.</td>
<td>155</td>
<td>9</td>
<td>86</td>
<td>2.27</td>
<td>N</td>
</tr>
<tr>
<td>15-Family problems such as alcoholism or marital disorder often contribute to a child’s ADHD.</td>
<td>174</td>
<td>6</td>
<td>70</td>
<td>2.41</td>
<td>+ve</td>
</tr>
<tr>
<td>16-Some children develop ADHD because they want attention. R</td>
<td>169</td>
<td>-</td>
<td>81</td>
<td>1.64</td>
<td>–ve</td>
</tr>
<tr>
<td>17-Students with ADHD are just as difficult to manage in the classroom as any student. R</td>
<td>160</td>
<td>1</td>
<td>89</td>
<td>1.71</td>
<td>N</td>
</tr>
<tr>
<td>18-ADHD children are at a higher risk of truancy and escaping. R</td>
<td>164</td>
<td>2</td>
<td>84</td>
<td>1.68</td>
<td>N</td>
</tr>
<tr>
<td>19-ADHD children need psychological support</td>
<td>161</td>
<td>1</td>
<td>88</td>
<td>2.29</td>
<td>N</td>
</tr>
<tr>
<td>20-ADHD children’s IQ is more than that of non-ADHD children</td>
<td>127</td>
<td>7</td>
<td>116</td>
<td>2.04</td>
<td>N</td>
</tr>
<tr>
<td>21-ADHD children experience more difficulties in their relations with their Colleagues</td>
<td>168</td>
<td>8</td>
<td>74</td>
<td>2.37</td>
<td>+ve</td>
</tr>
<tr>
<td>22-ADHD children should receive less homework than others</td>
<td>132</td>
<td>4</td>
<td>114</td>
<td>2.07</td>
<td>N</td>
</tr>
</tbody>
</table>

Grand mean | 1.93 | Assessment Neutral

Ass.= assessment, R=reverse, –ve=Negative (1-1.66), N=Neutral (1.67-2.33), +ve=Positive (2.34-3)
Table 2 shows that teachers had neutral attitude toward ADHD, the grand mean of scores was 1.93.

Table 3: distribution of teachers by mean score level of attitudes

<table>
<thead>
<tr>
<th>Level of assessment</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (1-1.66)</td>
<td>11</td>
<td>4.4</td>
</tr>
<tr>
<td>Neutral (1.67-2.33)</td>
<td>239</td>
<td>95.6</td>
</tr>
<tr>
<td>Positive (2.34-3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean and standard deviation 1.93±0.151

Table 3 shows that 95.6% of teachers had neutral attitude toward ADHD.

Table 4: ANOVA for teachers’ attitude with regard to their socio-demographic characteristics.

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.061</td>
<td>3</td>
<td>.020</td>
<td>.880</td>
<td>.452</td>
</tr>
<tr>
<td>Within Groups</td>
<td>5.675</td>
<td>246</td>
<td>.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.736</td>
<td>249</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.096</td>
<td>1</td>
<td>.096</td>
<td>4.204</td>
<td>.041</td>
</tr>
<tr>
<td>Within Groups</td>
<td>5.641</td>
<td>248</td>
<td>.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.736</td>
<td>249</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.026</td>
<td>1</td>
<td>.026</td>
<td>1.133</td>
<td>.288</td>
</tr>
<tr>
<td>Within Groups</td>
<td>5.710</td>
<td>248</td>
<td>.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.736</td>
<td>249</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.121</td>
<td>4</td>
<td>.030</td>
<td>1.319</td>
<td>.264</td>
</tr>
<tr>
<td>Within Groups</td>
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<td>245</td>
<td>.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.736</td>
<td>249</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monthly income</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.002</td>
<td>2</td>
<td>.001</td>
<td>.033</td>
<td>.968</td>
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<td></td>
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<td>Between Groups</td>
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<td>.010</td>
<td>.427</td>
<td>.653</td>
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<td>247</td>
<td>.023</td>
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<td>Total</td>
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<td>Class in teaching</td>
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<td>Between Groups</td>
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<td>.025</td>
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<td>Within Groups</td>
<td>5.685</td>
<td>247</td>
<td>.023</td>
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<tr>
<td>Total</td>
<td>5.736</td>
<td>249</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

F=Fisher, d.f= degree of freedom, no significant (P>0.05), significant (P≤0.05), high significant (P≤0.01)
The results reveal that there is a significant statistical difference in teachers’ attitude with regard to their gender, whereas no significant difference is found with regard to other socio-demographic variables at p ≤ 0.05 (table 4).

Discussion

According to the socio demographic characteristics of the teachers in table (1). The findings indicate that most of teachers at age (41-51) years. Youssef, Hutchinson, & Youssef (2015) found that the mean of teachers age was at 39 years;(10) Aly, Mohammed, & Ahmed, (2015) found that most of the teachers at age less than 35 years;(11) Al Moghamsi, & Aljohani (2018) found that (53.8%) of teachers at age (31-40) years (12) . This finding not consisted with the present study finding.

According to gender more than half of teachers were females. Youssef, Hutchinson, & Youssef (2015) found that females more than males. Aly, Mohammed, & Ahmed, (2015) found that (61.9%) of teachers were females (11). Khademi et al., (2016) found that 86.8% of teachers were females. (13) These result in same line with our finding. The finding of Amiri, Noorazar, Fakhari, Darounkolaee, & Gharehgoz, (2017) consistent with current finding (14) . They found that majority of teachers were females.

Regarding to the educational achievement of teachers, more than half of the sample had bachelor graduated and teach in class 4-6 in male students’ school. Aly, Mohammed, & Ahmed, (2015) found that most of teachers had high education level (11) . Khademi et al., (2016) found that (60.5%) of teachers had bachelor educational level (13) . Alfageer et al., (2018) found that (86%) of the teachers had bachelor and diploma degree and teaching pupils at grade sixth (15) . Al Moghamsi, & Aljohani (2018) found that (91.3%) of sample had bachelor educational level. (12) Khalil, Alshareef, & Alshumrani, (2019) found that (80.7%) of teachers had bachelor degree (16) . All these findings supported the present study results.

Regarding to teacher attitude about ADHD, the findings show that, they had neutral attitude toward ADHD, the grand mean was 1.93 in (table 2). Finally, majority of teachers had neutral level of attitude toward ADHD in (table 3). Youssef, Hutchinson, & Youssef (2015) found that the attitudes toward children with ADHD were generally positive, although most teachers felt children with ADHD should be taught by specialist teachers (10) . Aly, Mohammed, & Ahmed, (2015) found that 55% of teachers had positive attitude toward ADHD (11) . Khademi et al., (2016) found that 65.1% of teachers had neutral attitude about ADHD (13) . Alfageer et al., (2018) found that Most of the participants in current survey tend to have a positive attitude toward ADHD (15) . These finding is supported the present study result.

Amiri, Noorazar, Fakhari, Darounkolaee, & Gharehgoz, (2017) found that almost half of the teachers believed that the educational system plays a very important role in perpetuating the symptoms of ADHD, while nearly half believed that special schools are needed. Although almost one third of teachers believed that the behavior of children with ADHD is deliberate in nature, fewer believed that punishment is needed and even fewer blamed the children’s family for their behavior (14) . This finding is consistent with the present study.

Conclusions

The study concluded that majority of teachers had neutral level of attitude toward ADHD, and teachers’ attitude is affected by their gender.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References


Guidelines for Maintaining Physical Fitness During COVID-19 Pandemic

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Abstract
The ongoing corona virus outbreak (COVID-19) has turned into pandemic, by rapidly spreading and infecting all over the world. It left no other choice than implementing the lockdown all over the country. This lockdown has led to the development of sedentary lifestyle among the people leaving them with inactivity, anxiety, depression and with an elevated chance of a potential aggravation of the health problems. Therefore, not just modifying people’s lifestyles during quarantine and keeping an active lifestyle at home, it is very necessary for the overall population’s safety, but particularly for those with additional risk factors and the elderly to conduct daily physical indoor activities and exercises. The data was collected from the electronic databases. The review includes the quarantine population of all age group and gender. After reviewing the articles it was concluded that the multi-component full-body programs should be carried out by the population, including aerobic, strengthening, balancing and stretching exercises by modifying and utilizing both the space and material available. Doing physical exercise will help us to stay active during quarantine period so that after the end of the crisis we will be able to resume our work without lethargy.

Keywords: Covid-19; Inactivity; Pandemic; Physical activity; Quarantine; Sedentary lifestyle

Introduction
The Corona virus (Covid-19) was introduced in China (Wuhan) last December 2019, and the infection has spread worldwide (1). The World Health Organization, recognizing the worsening scenario, declared the rapidly spreading corona virus outbreak a pandemic (2). In last three months, the virus raced all over the world with more than 19,80,700 confirmed cases till 14th April 14, 2020, With 1,25,000 deaths, and over 4,65,700 worldwide recovered. Covid-19 infection is serious condition because it is highly and rapidly contagious (3).

In about 80 per cent of those infected, the virus causes mild respiratory infections, though about half of them will have pneumonia. A further 15 percent experience serious illness and a further 5 percent needs urgent treatment (2).

WHO officials recommended countries to implement a strategic combination of “containment and mitigation” (2). The former includes attempts to identify and avoid known transmission chains by isolating cases and tracking their connections, and possibly quarantining them. Mitigation involves steps at Community level such as social distancing and lockdown. Despite the fact that a quarantine period is the best choice and recommendation for stopping the rapid spread of infections, this may have collateral effects on other dimensions of the health of isolated patients, especially those listed as at higher risk (4)(those aged 65 years and those with serious heart disease, chronic lung disease, diabetes, obesity, and chronic kidney and liver disease) (5). The normal routine lifestyle of these people involved physical activity and exercise to ensure an adequate state of health (e.g. in diabetes, hypertension, CVS conditions) (1). Staying for a long time is likely to lead to sedentary behavior, such as
spending more time sitting or lying down for screening exercises, decreasing daily physical activity, leading to an increased risk for future health condition worsening. Therefore, changing people’s lifestyles during quarantine and maintaining an active lifestyle at home is very important for the health of the population as a whole, but particularly for those with additional risk factors and the elderly to perform routine indoor physical activities and exercises as going to be discuss further. There are some research articles published until now, but there was need to compare and summarize all the papers and make a cumulative guideline of all.

Methodology

Data source:- The data was collected from the electronic databases including, Science direct, Google scholar, Pub med, web of science ,etc. The review includes all the quarantine population of all age group and gender. The main focus was on the high risk population.

Discussion

Following the recommendations of the scientific societies, health institutions, and experts is of utmost importance. All the research articles suggest that physical exercise plays a beneficial role in the prevention of diseases, as an adjuvant treatment in chronic diseases and in psychological wellbeing. They also stated that physical exercises have protective effect on immune system especially in chronically ill patients\(^5,6\). Maintaining physical activity is the best way to counteract sedentary conduct and reduce the psychological effects of quarantine, as sedentariness has a well-known detrimental effect on cardiovascular function. In the present quarantine days, the available space and resources need to be modified and utilized.

In his paper, Rodriguez et al suggested conducting Multi component full-body programs like aerobics, strengthening, balance and power, mobility of coordination, and stretching exercises. They are directly related to the physiological activities of the main organs (respiratory, circulatory, muscular, nervous and skeletal systems) and are indirectly involved in the smooth functioning of other systems (endocrine, digestive, immune or renal systems)\(^7\).

Rodriguez et al, accurately summarized the guidelines of the major healthcare institutions for exercise which can be used as a physical fitness protocol\(^5\).

Physical fitness guidelines and recommendations

The indoor physical activities that can be perform during quarantine period are\(^8,9\):

Ø Cardio respiratory fitness exercise
- Walk briskly around the house
- Stair climbing
- Jogging or marching on the place
- Standing or walking while using phone
- Dancing for 15 minutes
- Skipping rope
- Cardio machine (if available)
- Online exercise routines
- Active videogames

Ø Resistance or muscular strengthening exercise
- Squats, sit-to-stand and stand-to-sit from a sturdy chair
- Push-ups to the wall
- Single-leg step-ups on stairs
- Alternating leg lunges
- Strength training video
- Sit-ups or crunches
- Weightlifting (e.g., dumbbells, bottles, packs)
- Exercises by resistance band, or clothes, belt, etc.

Ø As a balanced workout should incorporate pulling as well as pushing motions

The writers have brilliantly proposed an update of the international physical activity guidelines to the present situation. With the knowledge of exercise, it is also necessary to be well aware about the principle
elements of exercise program such as modality, frequency, volume, intensity, duration. In this regard, Jiménez-Pavón et al. performed a critical review of the most acceptable guidelines for elements of exercise, particularly those that address the elderly. He described the guidelines as

Exercise modality:- Multi component exercise program with previously stated elements including cognitive training for elderly.

Exercise frequency:- Recommended 5 days per week for older people, which could be increased to 5-7 days per week with volume and intensity adaptation in this particular quarantine situation.

Exercise volume:- It could be suggested under the quarantine to increase to 200-400 min per week distributed between 5-7 days to compensate for the decrease in the normal daily PA levels.

Exercise Intensity:- Moderate intensity (40-60 per cent heart rate reserve or 65-75 per cent maximum heart rate) should be the ideal choice for older people to enhance the protective role of exercise during quarantine times.

In terms of cardiovascular health, we agree with the statement that, “something is better than nothing”. Chen at al, said the objective should be to do at least 30 minutes of moderate physical activity every day and/or 20 minutes of vigorous physical activity every other day. Ideally we should use the combination of both intensities. Portable health gadgets simplified the quantification and monitoring of exercise routines. Nonetheless, due to the availability of many sites promoting physical activity, it is important to consult knowledge sources established by medical societies, physicians and sports health professionals in order to achieve optimum cardiovascular and skeletal muscle fitness, within the limits permitted by quarantine.

Conclusion

During this on-going COVID-19 pandemic, home stay is an inevitable fundamental safety step that can stop the widely spreading infection. But this prolonged home stay will lead to the development of sedentary behavior leading to inactivity, anxiety, depression which will ultimately lead to chronic health problem. Maintaining the regular physical habit and adapting the new ways of doing physical exercise will help us to stay active during quarantine period and after the end of the crisis we will be able to resume our work without laziness. The population should perform multi component full-body programs including aerobic, strengthening, balance, and stretching exercises.

Author’s Contribution: All authors contributed equally to the manuscript.

Funding: This study has not received any external funding.

Conflict of Interest: The authors declare that there are no conflicts of interests.

Ethical Clearance: An Ethical clearance was taken by the departmental research and ethical committee of Ravi Nair Physiotherapy College, Datta Meghe Institute of Medical Sciences, Sawangi(M) Wardha.

References


Effect of Hemodialysis on Some Biochemical Parameters in Diabetic Nephropathy Patients

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Abstract

Diabetes mellitus is a worldwide epidemic and is associated with long-term damage and causes dysfunction of several organs like kidney leading to diabetic nephropathy. This study was designed to evaluate some biochemical aspects.

This study was conducted on 95 patients which included (35) HD patients with diabetes and (35) HD patients without diabetes and (25) Type 2 diabetes mellitus patients, who attended at Ramadi General Hospital. For the purpose of comparison, (25) samples as control. Several tests were performed such as FSB, urea, creatinine, glomerular filtration rate (GFR), albumin, sodium ion, calcium ion, Phosphorus ion, potassium ion and magnesium ion were calculated. This study showed the level of urea and creatinine were a significant increase in HD patients with diabetes(128.29±22.59 and 7.1±1.76) mg/dl respectively, and HD patients without diabetes were (125.74±23.28 and 6.80±2.71) mg/dl respectively. The levels of Na⁺ and Ca++ were decrease in HD patients with diabetes (137.6±4.8 mmol/L and 6.72±1.76mg/dl) respectively. The levels of P, K⁺ and Mg++ were a significant increase in HD patients without diabetes(6.26±0.84 mg/dl, 5.52±0.61 mmol/L and 2.2±0.13mg/dl) respectively. The urea, creatinine and GFR are simple and useful biomarkers which can serve as predictor tests for assessing kidney functions in diabetic patients.

Key words: Chronic kidney disease (CKD), Diabetes mellitus (DM), Hemodialysis (HD), Sodium ion, Urea.

Introduction

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate leading persistent high level of blood glucose due to factors that oppose the action of insulin. Presently, DM is a worldwide epidemic and a great challenge to health care systems everywhere [1]. Chronic kidney disease (CKD) is a progressive loss of kidney function over a period of months or years through five stages or defined as decreased GFR and increased urinary albumin excretion. Each stage is a progression through a low and deteriorating glomerular filtration rate (GFR) [2]. Therefore, CKD a major global public health problem. Urea and creatinine are renal function markers indicating normal functioning of the kidney and increase of these substances in the serum indicate kidney dysfunction [3]. Patients with CKD have marked disruption in bone and mineral metabolism resulting in a complex disorder. The biochemical alterations of CKD-mineral bone disorder include elevated serum phosphate and decreased serum calcium [4]. The kidney is the route of potassium and sodium excretion from the body. Advanced renal failure typically results in potassium and sodium retention [5]. The tissue sodium accumulation has different pathophysiologic that may be amenable to therapeutic strategies in CKD patients. While low serum magnesium levels are associated with vascular calcification and increased cardiovascular mortality in CKD patients [6]. Aims of the study to measure and compare the levels of urea, creatinine, albumin, and GFR in study groups and to assess serum electrolytes levels in HD patients and T2DM patients.

Materials and Methods

The samples collection were started from April,
2019 till end of June 2019. The study is designed on 95 patients and (25) samples Control group. Patients divided into (35) hemodialytic patients with diabetes and (35) hemodialytic patients without diabetes and (25) T2DM patients. HD patient with diabetes and HD patient without diabetes samples were collected from the Industrial Renal department and T2DM patients from Diabetes Center at Ramadi General Hospital.

**Blood Samples Collection:**

Before the collection of samples, a careful history was taken from each patient according to a questionnaire and all patients provided written informed consent before participation in this study. From each patient and control, 5 ml of blood were obtained by venipuncture, the blood was dispensed in a gel plain tube and left for 30 minutes to clot at room temperature (18-25°C). Then, it was centrifuged at 3000 r.p.m for 10 minutes to collect serum, the serum were used in the estimation of FBS, Urea, Creatinine, Albumin, Na, Ca++, P, K, and Mg++.

**Biochemical tests:**

Determination of urea, creatinine, albumin, Na, Ca, P, K, and Mg as cited by manufacturing company kits which provided from human company / Germany. FBS determination according to [7] method, which provided with kit from linear company/ Spain.

**GFR Calculated:** According to [8] equation which is:

\[
\text{GFR}= 141^\times \text{min} (\text{Scr/k,1}) \times \text{max} (\text{Scr/K,1}-1.209 \times 0.993 \text{ Age} \times 1.018 \times 1.159 \times 1.018)\]

Scr: Serum creatinine mg/dl, K: is 0.9 man and 0.7 woman, \(\alpha\): -0.411 man and -0.329 women, Min: minimum of Scr/k and max indicates the maximum of Scr/ k.

**Statistical Methods:**

The data were translated into a computerized database structure, and the statistical analyses were carried out SPSS version 25. One way ANOVA test was used to find means and standard deviation (SD) for all variables of the study. The difference significances in proportions analyzed by LSD test, P-value less than 0.05 were considered to be significant.

**Results and Discussion**

Determination of serum Urea, Creatinine, GFR and albumin in four study groups:

This study showed that the FBS was a significant increase in diabetic group (215±11.1)mg/dl, followed by HD patients with diabetes (188.7±18.39)mg/dl. These findings showed mean of urea and creatinine were a significant increase in HD patients with diabetes (128.29±22.59, 7.1±1.76) mg/dl respectively and in HD patients without diabetes (125.74±23.28, 6.80±2.71) mg/dl respectively. While level of GFR was a significant decreased in HD patients with diabetes and HD patients without diabetes (8.22±1.69 and 9.93±1.47) mL/ min/1.73m\(^2\) respectively compared with control and diabetic patients (115.56±4.39 and 113.92±3.14) mL/ min/1.73m\(^2\) respectively. This study indicates the no significant difference in mean of the albumin in groups of the study, as shown in the Table (1).

**Table (1):** The difference in mean of FBS, urea, creatinine, GFR and albumin among four study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N.</th>
<th>Mean±SD of FBS NV(70-100) mg/dl</th>
<th>Mean±SD of Urea NV(10-50) mg/dl</th>
<th>Mean±SD of Creatinine NV(0.6-1.1)mg/dl</th>
<th>Mean±SD of GFR NV(120) mL/min/1.73m(^2)</th>
<th>Mean±SD of Albumin NV(3.81-4.65) g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>88.5 ± 4.88a</td>
<td>25.42±5.56a</td>
<td>0.76±0.14a</td>
<td>115.56±4.39a</td>
<td>4.2±1.76a</td>
</tr>
<tr>
<td>Diabetes</td>
<td>25</td>
<td>215 ± 11.1b</td>
<td>30.8±3.33b</td>
<td>0.98±0.34b</td>
<td>113.92±3.14a</td>
<td>4±1.14a</td>
</tr>
</tbody>
</table>
Cont.. Table (1): The difference in mean of FBS, urea, creatinine, GFR and albumin among four study groups.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD patients with diabetes</td>
<td>188.7±18.3c</td>
<td>128.29±22.5c</td>
<td>7.1±1.76c</td>
<td>8.22±1.69b</td>
<td>3.96±1.72a</td>
</tr>
<tr>
<td>HD patients without diabetes</td>
<td>92.2±2.7a</td>
<td>125.7±23.8c</td>
<td>6.80±2.71c</td>
<td>9.93±1.47b</td>
<td>3.92±1.11a</td>
</tr>
<tr>
<td>Total</td>
<td>146.1±26.3</td>
<td>78.57±78.54</td>
<td>4.1±3.22</td>
<td>58.44±45.81</td>
<td>3.952±2.18</td>
</tr>
</tbody>
</table>

*Different Letters (a, b, c): Means significant difference at P ≤0.05.
*Similar Letters: Means no significant difference at P ≤0.05.

These results agreed with [9; 10; 3; 11] who recorded that urea and creatinine levels increased in diabetic patients compared with control.

An increase in urea and creatinine levels and decreased GFR is seen when the kidney is not functioning properly. GFR is the best measure of kidney function since it accounts for age, BMI, and sex. Irrespective of its cause, kidney disease is associated with a decrease in GFR, and the severity of kidney disease correlates closely but inversely with GFR [3]. In this study, the HD patients showed higher levels of both urea and Cr compared to controls, as clear evidence for the microvascular abnormalities in the renal system. Elevations in urea occur as the number of functional nephrons decreases[12]. Creatinine is separated by the glomerulus; in this way, the creatinine level is an indirect measure of glomerular filtration. As GFR reduces, there is an ascent in the concentration of urea and creatinine[13]. In this study diabetic patients showed a significantly increase in urea and creatinine compared with control due to hyperglycemia in T2DM starts after the age of forty usually when the kidneys have already suffered the long-term consequences of aging and other promotors of chronic renal injury such as arterial hypertension, dyslipidemia, obesity. This probably might be a cause for increased levels of serum creatinine and urea in T2DM [9]. The inflammatory process is the most widely recognized cause of decreased concentration of albumin, and since DM was known to be an express provocative, this may be one reason that causes the observed decline in Albulmin concentration to occur [14].

Determination of serum Na, Ca++, P, K and Mg++ among four study groups:

This study indicates that mean of Na was a significant decrease in HD patients with diabetes and HD patients without diabetes (137.6±4.8 and 136.6±5.1) mmol/L respectively compared with control (140.75±1.84) mmol/L. The results indicate that mean of Ca++ is a significant decrease in HD patients with diabetes (6.72±1.76) mg/dl and HD patients without diabetes (6.88±1.59)mg/dl compared with control (9.72±0.63) mg/dl. Where P level was a significant increase in HD patients without diabetes and HD patients with diabetes (6.26±0.84 and 6.11±0.75) mg/dl respectively compared with control (4.22±0.67) mg/dl. While K level was a significant increase in HD patients with diabetes and HD patients with diabetes (5.59±0.7 and 5.52±0.61) mmol/L respectively. These results indicate Mg++ level was a significant increase in HD patients without diabetes and HD patients with diabetes (2.2±0.13 and 2.18±0.27) mg/dl respectively compared with diabetic patients and control, as shown in the Table (2).
Table (2): The difference in mean of Na, Ca++, P, K and Mg++ among four study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N.</th>
<th>Mean±SD Na (135-155) mmol/L</th>
<th>Mean±SD Ca++ (8.1-10.4) mg/dl</th>
<th>Mean±SD P (2.5-5) mg/dl</th>
<th>Mean±SD K (3.6-5.5) mmol/L</th>
<th>Mean±SD Mg++ (1.9-2.5) mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>140.75±1.84a</td>
<td>9.72±0.63a</td>
<td>4.22±0.67a</td>
<td>4.31±0.8a</td>
<td>1.93±0.15a</td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>25</td>
<td>139.8±1.15a</td>
<td>9.04±0.5b</td>
<td>4.5±0.41a</td>
<td>4.12±0.79a</td>
<td>1.99±0.14a</td>
</tr>
<tr>
<td>HD patients with diabetes</td>
<td>35</td>
<td>137.6±4.8b</td>
<td>6.72±1.76c</td>
<td>6.11±0.75b</td>
<td>5.59±0.7b</td>
<td>2.18±0.27b</td>
</tr>
<tr>
<td>HD patients without diabetes</td>
<td>35</td>
<td>136.6±5.1b</td>
<td>6.88±1.59c</td>
<td>6.26±0.84b</td>
<td>5.52±0.61b</td>
<td>2.2±0.13b</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>138.37±4.62</td>
<td>7.89±4</td>
<td>5.42±4.20</td>
<td>4.29±0.83</td>
<td>2.01±0.67</td>
</tr>
</tbody>
</table>

*Different Letters (a, b, c): Mean significant difference at P ≤0.05.

These results correspond with [15; 16] who recorded sodium level was decreased in HD patients compared with control. These findings agreed with [11; 17; 18] who recorded that the Ca++ showed significant decrease in HD patients, while phosphorus and K were increased in HD patients compared to control. These results no correspond with [6] who found that the mean Na+ was increased, while mean of phosphorus, Mg and potassium were decreased in HD patients compared with control.

CKD is a catabolic state and is associated with progressive nephron destruction and miss of renal function decreases the body ability to release a sodium load, which causes extracellular volume enlargement and sodium retention, and as such, the restoration of sodium balance is one of the key objectives of HD treatments [19]. Where in chronic HD patients, Na+ balance largely depends on interdialytic dietary salt intake and intradialytic Na+ removal[5].

Moreover, it is now recognized that increased sodium intake does not necessarily result in increased urinary sodium excretion and that sodium can exchange for intracellular potassium and accumulate in tissue matrix without any corresponding fluid status changes, the so-called non-osmotic sodium balance [20]. Ca++ plays an important role in the regulation of glucose level in the blood particularly postprandial glucose level, hence Ca++ should be measured in patients with T2DM who have uncontrolled hyperglycemia. There is evidence to suggest that altered Vit D and Ca++ homeostasis may play a role in the development of T2DM [11]. The reduction in serum Ca++ levels were most likely due to several factors: Reduction in insulin levels that impair bone formation due to stimulation of osteoblast proliferation and impairment of Ca++ homeostasis; Hyperglycemia, which increases the excretion of Ca++ and phosphorus in urine [21]. In this study, the serum Ca++ levels decreased in HD patients with/ without diabetes because the serum Ca++ at dialysis initiation to be associated with all-cause mortality after dialysis initiation. Severe hypocalcemia is highly likely to occur during the early period after dialysis initiation [17].

In our results, we observed that the mean of phosphorus was increased in HD patients with/ without diabetes compared with control and diabetic patients the usual cause is a decrease in renal excretion of phosphate. Advanced renal insufficiency reduces excretion sufficiently to increase serum phosphor [18]. At later CKD stages, the hyperphosphatemia occurs either by increasing bone reabsorption and phosphate release or by reducing the bone formation and phosphate uptake[22].
HD patients present with hyperkalemia, which reduces the resting membrane potential, slows the conduction velocity and increases the rate of repolarization [23]. All these changes are the signs of membrane instability and cardiac arrest or ventricular fibrillation may follow and thus this situation usually requires careful and prompt management [24]. During hemodialysis, there is a quick shift of serum K+ which leads to hypokalemia after HD sessions [25]. This study did not show an association between serum Mg++ levels and a risk of incident T2DM in patients with T2DM, compared to controls these slight differences may be supplements drugs or maybe a compensatory state [11]. The young erythrocytes have a higher Mg++ concentration than older cells for this reason in patients undergoing HD have a higher Mg++ concentrations and have been shown to be dependent on residual renal function [26].

**Conclusion**

GFR, urea and creatinine are simple and useful biomarkers which can serve as predictor tests for assessing kidney functions in diabetic patients. The serum Mg, P and K were increased in HD patients, these a worthwhile tool in assessing duration of disease, morbidity and mortality in HD patients. They estimation may help in evaluating conservative treatment and dialysis in CKD. Decreased levels of Na and Ca in HD patients compared with control group.

**Conflict of Issue - Nil**

**Ethical Clearance – Obtained.**

**Fund – Self.**

**References**


Submaximal Exercise Task Post Clinical Balance in COPD Patients and its Correlation with Quadriceps Girth, Body Mass Index and Disease Severity

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Abstract

Purpose: To study and compare the effect of submaximal exercise on static and dynamic balance in patients with severe and moderate chronic obstructive pulmonary disease (COPD) and correlation of the balance impairments with disease severity, quadriceps girth and body mass index (BMI).

Methods: A sample of convenience of 45 middle aged participants (mean age 53.47 ± SD 4.775), including 15 with severe COPD, 15 with moderate COPD who were not undergoing pulmonary rehabilitation program and 15 healthy controls were included. The subjects performed Timed Up and Go Test (TUG) for dynamic balance and quiet standing to assess postural sway using sway meter with eyes closed and opened in narrow, semi tandem and tandem stance for 30 seconds. The balance variables were then correlated to forced expiratory volume in one second (FEV1), quadriceps girth and body mass index. Significance was set at alpha less than 0.05.

Results: when compared to healthy controls, significant differences were found in postural sway in all the stances in severe COPD patients, and in semi tandem and tandem stances in moderate COPD patients. TUG test significantly differed in both severe and moderate COPD (p=0.0001) and no difference between severe and moderate COPD TUG test was found. Moderate negative correlation was found between FEV1 and postural away in few stances whereas no correlation was found with quadriceps girth and BMI of COPD patients.

Conclusion: Static as well as dynamic balance are affected in COPD patients with postural sway affected more in severe COPD than moderate COPD leading to functional disability and risk of falls which increases with increase in age. Balance alterations can be moderately correlated to FEV1.

Keywords: Chronic Obstructive Pulmonary Disease, submaximal exercise, state and dynamic balance, anteroposterior sway, mediolateral sway, total sway, Timed Up and Go test, Quadriceps girth, Body Mass Index, FEV1.

Introduction

The detriments of peripheral muscle weakness have been noted in chronic obstructive pulmonary disease (COPD) along with hypoxia and hypercapnia, due to systemic manifestations. This extra pulmonary manifestation of has found to be associated with reduced functional mobility and exercise tolerance and deficits in...
simple motor movement\textsuperscript{1,2}. Thus, along with exertional dyspnea being the main factor for functional limitation, peripheral muscle strength also contributes for the same, lower limb being more affected than upper limb\textsuperscript{1}. Quadriceps myopathy is the most recognized characters of COPD coma resulting to two functional dysfunction which is a result of systemic inflammatory response and inactivity\textsuperscript{3,4}. Some studies have shown that myopathy is known to contribute to neurological abnormalities in patient in critical care unit and also that hypoxia effects motor coordination and postural control at high altitude and does they may have a similar effect in patient with hypoxic COPD\textsuperscript{1,5,6}.

Balance is an important component for performing activities of daily life and for being functionally independent. Recent evidences have stated that balance impairment is observed in elder COPD patients. Even balance assessment proved effective in the fallers from non-fallers in elder COPD. Evidence have stated that static balance is affected serious stable COPD following submaximal exercise task. But no studies have been performed to know the differences between moderate and severe COPD and how far quadriceps girth which has found to be reduced in COPD patients due to god resets myopathy is related to balance. Few researchers have studied the effect of body mass index BMI on postal balance but not in BD population. The aim of this study is to compare static and dynamic balance of senior and moderate COPD patients and to correlate it with quadriceps girth, BMI and forced expiratory volume in one second (FEVI) in middle age see OPD so that some of the aging effects on balance can be excluded and balance impairments can be well associated with the disease condition.

**Methods**

45 participants falling in the age group of 45 to 60 years (mean age 53.47± SD 4.775) were recruited including 15 with stable severe COPD (mean FEVI =41.27), 15 stable moderate COPD mean FEVI= 68.33) and 15 healthy controls from the outpatient department of Lala Ram Swaroop TB and Respiratory Medicine Institute. Patients were excluded if they had a history of major exacerbation in past one month. Major orthopedics condition, mobility limiting arthritis, neuromuscular condition\textsuperscript{1,2}, unstable angina, myocardial infarction and hypertension\textsuperscript{8}, any pain (either localized or general) or any conditions affecting low back or lower limb\textsuperscript{7}, cognitive impairment\textsuperscript{1}, vestibular disorders\textsuperscript{1,7}, tuberculosis, other lung diseases other than COPD\textsuperscript{1,7}. A through explanation of the procedure was performed and possible risks and benefits were explained. Written informed consent was obtained from each participant, with ethical clearance from the Research Ethical Committee of Lala Ram Swaroop Institute of TB and Respiratory Care, New Delhi.

**Measurement:**

The pre exercise baseline measurement of breathlessness using Brogs Dyspnoea scale, baseline oxygen saturation and baseline heart rate was measured after enrolling the participant in the study. Quadriceps girth was also measured prior to the test using cloth measuring tape taking lateral joint as a point from which girth was measured at two different levels 10 cm and 20 cm for both the limbs\textsuperscript{9}. Static balance was measured by assessing postural sway in the three validated stances, narrow, semitandem and tandem\textsuperscript{10} i.e. with increasing level of difficulty, with both eyes open and eyes closed using an instrument called “Swaymeter”. It consists of a pair of graduated ruler’s oriented perpendicular to each other and fixed upon a stand. It measures the displacement of the body at the level of the waist in anteroposterior, mediolateral direction and total sway in all the stances with the increasing level of their difficulty through the pen attached to a stand together, which is fixed with a belt\textsuperscript{11}. Patient were asked to stand for 30 seconds in narrow stance with both the feet together, semi tandem stance with the big toe of one foot placed at the side of the heel of the other foot and tandem stance with the big toe of one foot touching the heel of the foot placed ahead of it.

TUG test was performed to assess dynamic balance as well as functional mobility. Subjects were instructed to start by sitting on a chair, which was kept constant (46cm) for all the patients and on the word go to stand up, walk 3m, turn around, walk back to the chair, and sit down. Time was calculated from the time the pelvis was lifted to the time it was placed back on the chair. For older adults, completing the task in 7 to 9 seconds is low risk of hampered dynamic balance which may lead to falls, moderate risk in 10 to 12 seconds, and high risk...
in 13 seconds or more. The order of balance measures was randomized full stop the measurements obtained through the procedure was then correlated to quadriceps girth, FEVI and body mass index of the patients with COPD in order to obtain the relationship between balance and these variables.

**Statistical Analysis**

Analysis was performed using SPSS software version 15.0. the paired t test was applied for within subject dependent variable. As multiple groups were taken, a post hoc analysis was performed using tukey test to compare the difference between each reading. An alpha value of 5% was set for finding the significant difference. Pearson’s r correlation analysis was used to determine the correlation of quadriceps girth, BMI and FEVI with static and dynamic balance.

**Results**

The demographic characteristics of the patients are presented in table 1. All three groups did not differ significantly with respect to age. The Spirometry results showed significant difference in FEV1 among all the three groups. Dyspnea increased on 20 point Borg scale to 10.78 +-2.476 post task as compared to pre task mean rating of 8.84 +- 2.335.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe COPD</th>
<th>Moderate COPD</th>
<th>Healthy Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Yrs</td>
<td>54.87 ± 4.984</td>
<td>53.27 ± 4.480</td>
<td>51.85 ± 5.04</td>
<td>53.47 ± 4.775</td>
</tr>
<tr>
<td>BMI</td>
<td>19.3 ± 1.70</td>
<td>18.97 ± 2.374</td>
<td>24.76 ± 2.421</td>
<td>21.573 ± 3.494</td>
</tr>
<tr>
<td>QG</td>
<td>31.93 ± 1.981</td>
<td>33.73 ± 3.081</td>
<td>40.85 ± 1.908</td>
<td>35.6 ± 4.658</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>41.27 ± 6.33</td>
<td>68.33 ± 8.764</td>
<td>95 ± 5.099</td>
<td>67.51 ± 23.6</td>
</tr>
</tbody>
</table>

| Effect of exercise on TUG test: |

Significant differences was found between severe and healthy control (p =0.0001) and between moderate and healthy controls (p =0.0001). No difference was found in tea UG performance between severe and moderate COPD (p=0.4).

| Effect of exercise on postural sway: |

<table>
<thead>
<tr>
<th>Table 2: Post Hoc Tukey Analysis results severe COPD to healthy controls in marrow, semitandem and tandem stance in eyes closed AP, ML and total sway.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Prenarrowecap</td>
</tr>
<tr>
<td>postnarrowecap</td>
</tr>
</tbody>
</table>
**Eyes open:**

Mean increase in anterior posterior by 1.05, mediolateral (ML) by 2.15cm as well as total sway by 3.22 cm was found in narrow stance in severe COPD patients. Mediolateral as well as total sway was found to be affected in all the remaining stances in COPD patients given in table 2, 3, 4, 5. Mediolateral (p=0.0001) and total sway was found to be affected in moderate COPD patients in tandem stance (p=0.024). Healthy controls also show significant difference in mediolateral sway (p=0.004) and total sway (p=0.02) in tandem stance.

**Eyes closed:**

According to the post hoc tukey analysis, severe COPD patients showed increase anteroposterior sway, mediolateral sway and total sway in all narrow, tandem and semi tandem stances as compared to healthy controls (table 2, 3, 4, 5). However moderate COPD patients showed increased mediolateral sway in semi tandem stance (p=0.003) and anteroposterior sway (p=0.001) and mediolateral sway in tandem stance (p=0.0001) significant difference in total sway in tandem stance (p=0.0001) was also found in moderate COPD patients.

<table>
<thead>
<tr>
<th>Table 3: Narrow Stance</th>
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<tbody>
<tr>
<td><strong>Severe</strong></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
</tr>
<tr>
<td>Eyes Open Total Sway</td>
</tr>
<tr>
<td>Eyes Closed AP Sway</td>
</tr>
<tr>
<td>Eyes Open in AP Sway</td>
</tr>
<tr>
<td>Eyes Open in ML Sway</td>
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<tr>
<td>Eyes Closed ML Sway</td>
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Table 4: Semi tandem Stance

<table>
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<th></th>
<th>Moderate</th>
<th></th>
<th>Healthy</th>
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</thead>
<tbody>
<tr>
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<td>pre</td>
<td>post</td>
<td>pre</td>
<td>post</td>
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<tr>
<td>Eyes Open Total Sway</td>
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<tr>
<td>Eyes Closed AP Sway</td>
<td>8.7 cm</td>
<td>9.5 cm</td>
<td>7.0 cm</td>
<td>8.0 cm</td>
<td>6 cm</td>
<td>6 cm</td>
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<tr>
<td>Eyes Open in AP Sway</td>
<td>4.0 cm</td>
<td>4.5 cm</td>
<td>3.5 cm</td>
<td>4.5 cm</td>
<td>3 cm</td>
<td>3.5 cm</td>
</tr>
<tr>
<td>Eyes Open in ML Sway</td>
<td>5.2 cm</td>
<td>6.0 cm</td>
<td>3.7 cm</td>
<td>4.5 cm</td>
<td>3.5 cm</td>
<td>3.5 cm</td>
</tr>
<tr>
<td>Eyes Closed ML Sway</td>
<td>9.0 cm</td>
<td>1.0 cm</td>
<td>8.0 cm</td>
<td>9.0 cm</td>
<td>5.0 cm</td>
<td>5.0 cm</td>
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<tr>
<td>Eyes Closed in Total Sway</td>
<td>11 cm</td>
<td>19.5 cm</td>
<td>10 cm</td>
<td>16 cm</td>
<td>9.0 cm</td>
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</table>

Table 5: Tandem Stance

<table>
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<th></th>
<th>Moderate</th>
<th></th>
<th>Healthy</th>
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<tr>
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<td>post</td>
<td>pre</td>
<td>post</td>
<td>pre</td>
<td>post</td>
<td></td>
</tr>
<tr>
<td>Eyes Open Total Sway</td>
<td>15 cm</td>
<td>19 cm</td>
<td>16 cm</td>
<td>18 cm</td>
<td>10.2 cm</td>
<td>10.8 cm</td>
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<tr>
<td>Eyes Open in AP Sway</td>
<td>4.8 cm</td>
<td>5.5 cm</td>
<td>5.0 cm</td>
<td>6.0 cm</td>
<td>3.5 cm</td>
<td>3.5 cm</td>
</tr>
<tr>
<td>Eyes Open in ML Sway</td>
<td>8.2 cm</td>
<td>10 cm</td>
<td>7.7 cm</td>
<td>8.5 cm</td>
<td>6.5 cm</td>
<td>6.5 cm</td>
</tr>
<tr>
<td>Eyes Closed in Total Sway</td>
<td>21 cm</td>
<td>22 cm</td>
<td>18 cm</td>
<td>20.2 cm</td>
<td>10 cm</td>
<td>10 cm</td>
</tr>
</tbody>
</table>

Correlation analysis:

Pearson’s correlation showed moderate negative correlation was obtained FEV1 and postural sway in four stances ML sway in eyes open (r = -0.473, p = 0.008) and closed (r = -0.394, p = 0.03) in narrow stance, semi tandem and tandem stance (r = -0.522, p = 0.003). Correlation analysis was done within 30 COPD patients. No significant correlation was obtained between quadriceps girth, BMI and balance.

Discussion

The purpose of this study was to assess and compare the effects of submaximal exercise task on postural balance, including both static and dynamic balance in COPD patients as compared to healthy controls in the middle aged individuals and to correlate the balance with FEV1, Quadriceps girth and Body Mass Index. The results found significant differences in dynamic balance of severe and moderate as compared to healthy individuals. Total postural sway was found to be affected from normal to challenging stances in severe COPD patients whereas mediolateral sway was only found to be affected in moderate COPD in challenging stances like semi tandem and tandem with more significant differences found in eyes closed stances than eyes open ones. Moderate negative correlation was found between FEV1 and postural sway in four of the total stances indicating inverse relationship between FEV1 and postural sway. This study was design to compare the balance between moderate and severe COPD in middle age population so that some of the effects of aging on
balance which is usually found above 60 years, based on the evidences\textsuperscript{13–16}, can be excluded.

Simple inexpensive, reliable and valid test like TUG test\textsuperscript{7,17–19} for dynamic balance and postural sway through sway meter\textsuperscript{7,11,20} were used to assess balance which can be easily and economically available and used in clinical settings. The alteration in balance as interpreted from the study, can be considered as a consequence of either muscle fatigue which would have occurred due to an exercise task\textsuperscript{7,21,22}. Increase in sway especially mediolateral has been reported in challenging positions in normal individual\textsuperscript{7}, however according to the findings of this study COPD were found to have alterations in balance in even in normal narrow stance and less challenging semi tandem stance as well as showed increased time to complete TUG test. One of the reasons for this may be Quadriceps myopathy since noticeable decreased girth was found in both severe and moderate COPD as compared to healthy controls. Evidence have stated that weak quadriceps muscle is related to postural imbalance and leads to increase postural sway which poses the risk of falls\textsuperscript{13,20}. But since the fatigue and muscle strength was not assessed in this study, and no significant correlation was obtained between girth and balance, it cannot be clearly explained from this point of view.

After contributing factor for an altered balance can be breathlessness since evidences have stated that hypoxia can lead to altered postural balance\textsuperscript{5,6}. According to Killian and Campbell\textsuperscript{23}, as COPD progresses, respiratory muscles have to generate increased pressures to maintain an adequate ventilatory thresh hold resulting in increased dyspnea. Few evidences have shown that myopathy is known to contribute to neurological abnormalities in patient in critical care units and you have stated that hypoxia effects motor coordination and postural control at high altitudes and does they may have a similar effect in patient with hypoxic COPD\textsuperscript{1,5,6}. Even the disease severity it was found to be significantly correlated to postural sway in few variables in this study and evidences signifies the relationship between disease severity and postural balance\textsuperscript{1,24}. More clear results would have obtained if large sample size was taken for correlation. Does the reason for altered postural control in COPD can be thought to be related to disease severity as well as consequences of pulmonary and systemic manifestation in leading to hypoxia and quadriceps myopathy which needs to be further assessed using reliable and accurate measures.

Balance impairment: risk of fall and disability.

Studies have reported that increased CWE is associated with increased fall risk in an individual\textsuperscript{13,25}. Maki and lagoie et al has stated that increased lateral sway is the single best predictor of future following risk in his study on elder population\textsuperscript{16,26}. However peterka et al in his study of subjects ranging from 7 to 81 years stated that patient greater than 55 years shows significant greater and posterior sway which poses them to risk of fall\textsuperscript{14}. Similar findings were found by laughton et al\textsuperscript{26,27} who stated that followers displayed increased a peace way full stop in the study done by Angela at al and Scott et al in COPD patients increased mediolateral and total survey was demonstrated which was considered to hamper the activities of daily life of PD patients and causes them to the risk of falls\textsuperscript{1,7}. Beauchamp et al performed a study to discriminate followers from no followers in using balance measures and found that followers have altered static and dynamic balance the current study supports these findings indicating functional this ability and risk of falls are associated with COPD which increases with CVR 80 of the disease\textsuperscript{25}. Thus the key findings of this study show that severe COPD patients are more affected and have greater balance impairments than moderate COPD patients as compared to healthy controls this can hamper their functional activities and can lead to functional disability. Even moderate see OPD faces balance issues not as significant as severe ones, but are post to risk of falls and functional ability to sense lateral balance is found to be affected during challenging activities. TUG test that assess dynamic or walking balance was found to be significantly affected in both the groups and was falling in the range of high risk of fallers with a mean of greater than 16 seconds for both groups\textsuperscript{28}. This altered dynamic balance reflects a reduced ability while maintaining a standing posture and while performing a potentially destabilizing and challenging activities\textsuperscript{11}.

Limitations:

Use of force plates to record center of pressure excursions or posturography techniques can give more accurate result for the assessment of balance since the
readings obtained from Swaymeter was more manual though it has been validated against force plates. Small sample size and convenient sampling used was another limitation in the study.

Future studies can be done correlating quadriceps strength and endurance in COPD patients with balance. Balance impairment was only assessed in this study, but the time for which these balance alteration remains following an exercise task can be assessed in future research which can help in exercise prescription. Further research can be done to assess the effect of balance training in COPD.

**Conclusion**

Static as well as dynamic balance are affected in COPD patients with postural sway affected from normal to challenging stances in severe COPD patients. Moderate COPD patients also faces balance deficits but in more challenging and destabilizing conditions as compared to severe ones. Thus balance impairments should be assessed while enrolling the COPD patient pulmonary rehabilitation program to control the factors like disability, falls etc. associated with balance deficits. Balance alterations may be inversely related to FEV1.

**Informed patient consent:** Participants recruited in the study signed the informed patient consent forms, and these forms are secured securely at the site of the study.

**Author’s contribution:** All the authors led to the creation and design of the study. All the authors read and approved the final manuscript for publication.

**Conflict of Interest:** The authors declare no competing interests.

**Funding Support:** None.

**Acknowledgment:**

I wish to thank my guides, my teachers, all my friends and associates and most importantly my parents for their guidance, advice and support throughout the project period.

**References**


Evaluation the Effect of Disclusion time Reduction (DTR) in Management Myofacial Pain Syndrome in Iraqi Patients Using the Digital Occlusal Analysis (T-scan)

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¹Student, B.D.S Msc. ²Prof. College of Dentistry, University of Baghdad, Iraq

Abstract

The aim of the study is to evaluate the effectiveness of disclusion time reduction in treatment of myofacial pain syndrome using immediate complete anterior guidance development (ICAGD) protocol monitored by digital occlusal analysis (T-scan). Methods and materials: Subjects with full dentition and angle class I relation. DC/TMD criteria were used to diagnose the patients with various MFDS symptoms including mouth opening (assisted and unassisted), excursion movements (mediotrusion and protrusion) and painful masticatory muscles involvement. A visual analog scale was used to assess the pain intensity. Disclusion Time Reduction (DTR) of all molars and premolars to < 0.5 second on all studied locations include: (Centric relation, Maximum intercaspation, Right lateral, left lateral and protrusion) have been made, the patients were recalled tow times at the 7th day and 14th day for the follow up and the subjects were assessed for the symptoms relief. Results: Highly significant difference P≤ 0.001 in period (1st day pretreatment -7th day post treatment) and(1st day pretreatment- 14th day post treatment) related to changes in disclusion time and all symptoms of MFDS but in period (7th day post treatment -14th day post treatment) ,the data outside the comparison limited (OCL) was stated according to the pain intensity, mouth opening (assisted and unassisted)and excursion movements while a non- significant difference P>0.05 were documented related to changes in disclusion time and painful masticatory muscle involvement . Conclusion: The results showed the DTR less than 0.5 seconds reduces musculoskeletal-based symptoms of MFDS patients, and this method can be used clinically with highly success in treatment MPDS.

Keywords: Myofascial pain dysfunction syndrome (MFDS), Disclusion Time Reduction (DTR), Disclusion time (DT), immediate complete anterior guidance, Development (ICAGD).

Introduction

Myofascial pain dysfunction syndrome (MFDS) a common term utilized in dental medicine to depict orofacial chronic pain, is a functional disease related to the masticatory muscles, the neural structures, and the temporomandibular joint (TMJ) structures (1). It is characterized by trigger points affecting more than one muscle group and pain associated with muscle spasm and tenderness caused by touching these points (2). The etiology of TMD, especially muscle pain, is multifactorial and includes parafunctional habits, trauma, stress, heredity and occlusal factors (3). Several treatments have been suggested to control pain and symptoms. These include orthopedic stabilization, intraoral devices, and medications (4).

Good occlusion with symmetric occlusal contacts can influence the stability of orthognathic treatment. The number, location, and size of occlusal contacts, as well as the forces applied, are important for good functioning of the TMJ system (5). So Disclusion time reduction (DTR) is an objective treatment protocol using T-Scan (digital analysis of occlusion) for treating occlusally activated orofacial pains. Chronic occluso-muscle disorder is a myogenous subset of temporomandibular disorder symptoms (6).

Methods and Materials

This study was designed and conducted from April 2019 to February 2020 in the department of oral medicine in the teaching hospital of College of Dentistry/ University
of Baghdad. After obtaining ethical approval from the institutional ethical committee and written informed consent, the participants were selected according to the Diagnostic Criteria for Temporomandibular Disorders (7). The participants completed a self-reported questionnaire, containing information about personal, medical, and dental histories. Subjects with full dentition Angle class I relation included in this study. At each study visit, subjects were asked to answer a questionnaire about the current status of their symptoms according to mouth opening (assisted and unassisted), excursion movements (mediotrusion and protrusion) and painful masticatory muscles involvement. Also the pain intensity was evaluated according the ordinal number scale (VAS) to describe the current status of their condition.Before commencing any treatment occlusal adjustments, the Force Movie mode of the T-Scan was utilized to measure and calculate the pre-treatment Disclusion Times in seconds. After analyzing the pretreatment Disclusion Time data of each subject by digital occlusal analysis T-scan (Novous) Figure (1). The ICAGD enameloplasty (reduction) was performed so established immediate posterior disclusion of 0.5 seconds per each movement figure (2) and figure (4). Additionally, the subject was specifically asked if their new occlusion appeared to feel ‘noticeably lighter’ posteriorly. Each five movements (Centric relation, Maximum intercaspation, Right lateral, left lateral and protrusion) recorded two times in 7th day and 14th day post treatment with (DTR) to obtain a Mean values for each movement per subject. Later compared between the values in 1st day pre-treatment, 7th day post treatment and 14th day post treatment.

Analysis of data was carried out using the available statistical package of SPSS-25. The significance of difference of different means (quantitative data) were tested using Paired-t-test for difference of paired observations (or two dependent means. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test ($c^2$-test) with application of Yate’s correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.
Eighty five subjects (68 female and 17 male) with myofacial pain syndrome were included in this study.(25) patients their age range (18-43) years old had full dentition and angle class I have been treated with DTR therapy.

According to the effectiveness of DTR in different locations of movements a highly significant difference $P \leq 0.01$ documented in (1st day pre - 7th day post) treatment , non-significant difference $P > 0.5$ showed in period (7th post -14th day post)treatment and highly significant difference was stated in period (1st day pre -14th day post) treatment.( Table 1) showed Summary statistics concerning studied Measurement of Disclusion time relative to the effectiveness of DTR therapy in different locations of movements.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time interval (day)</th>
<th>Test (*) Statistics</th>
<th>Locations</th>
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<tr>
<td></td>
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<td>NS</td>
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<td>(1st pre-14th post)</td>
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<tr>
<td></td>
<td></td>
<td>P-value*</td>
<td>HS</td>
</tr>
</tbody>
</table>

(*) HS: High Significant at $P < 0.01$; NS: Non Significant at $P > 0.05$; DTR: Disclusion Time Reduction; MD: Mean Difference; MIC: maximum intercapsation;1st pre: first day pretreatment;7th post:7 day post-treatment;14th post:14th day post treatment.
100% patients were registered according to pain intensity pretreatment but only 10% (mild pain) was stated 7th day after treatment and this percentage remain constant to 14th day after treatment, so a highly significant difference \( \text{P} \leq 0.01 \) were registered in periods (1st day pre -7th day) and (1st day pre-14th day post) treatments but the data outside comparison limited a was reported in period (7th day -14th day) post treatment. Table (2)

**Table (2) Summary statistics concerning studied “Pain intensity” in relative to effectiveness of DTR therapy.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Statistic Time interval pain involvement</th>
<th>mild</th>
<th>Moderate</th>
<th>sever</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day pre</td>
<td>45</td>
<td>32</td>
<td>23</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>7th day post</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>14th day post</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

\%: percentage; HS: High Significant at \( \text{P} \leq 0.01 \); NS: non-Significant at \( \text{P} > 0.05 \); OCL: Outside comparison limited ; 1st pre: first day pretreatment; 7th post: 7 day post-treatment; 14th post: 14th day post treatment.

A MFDS symptoms according to painful masticatory muscles involvement appeared a highly significant difference \( \text{P} \leq 0.01 \) in period (1st day pretreatment - 7th day post-treatment) and (1st day pretreatment -14th day post-treatment) while non-significant difference \( \text{P} > 0.5 \) stated in period (7th day post-treatment -14th day post-treatment). Table (3)
Table (3) Summary statistics concerning studied Measurement “involvement of painful Muscles of Mastication” in relation to effectiveness of DTR therapy

<table>
<thead>
<tr>
<th>DTR muscles</th>
<th>pre -7th post</th>
<th>7th post-14th post</th>
<th>pre -14th post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle + Anterior Temporalis</td>
<td>0.0001 HS</td>
<td>0.007 HS</td>
<td>0.777 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.777 NS</td>
</tr>
<tr>
<td>Middle Temporalis</td>
<td>0.0001 HS</td>
<td>0.003 HS</td>
<td>0.542 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.647 NS</td>
</tr>
<tr>
<td>Middle + Posterior Temporalis</td>
<td>0.0001 HS</td>
<td>0.007 HS</td>
<td>0.867 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.647 NS</td>
</tr>
<tr>
<td>Middle masseter</td>
<td>0.0001 HS</td>
<td>0.0001 HS</td>
<td>0.867 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.867 NS</td>
</tr>
<tr>
<td>Middle + Superior masseter</td>
<td>0.0001 HS</td>
<td>0.0001 HS</td>
<td>0.350 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.647 NS</td>
</tr>
<tr>
<td>Middle + Inferior masseter</td>
<td>0.0001 HS</td>
<td>0.0001 HS</td>
<td>0.127 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.849 NS</td>
</tr>
<tr>
<td>Superior masseter</td>
<td>0.0001 HS</td>
<td>0.0001 HS</td>
<td>0.647 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.521 NS</td>
</tr>
</tbody>
</table>

(* ) HS: High Significant at P<0.01; NS: Non Significant at P> 0.05; DTR: Disclusion Time Reduction.; 1st pre: first day pretreatment; 7th post: 7 day post-treatment; 14th post: 14th day post treatment.

All the mean values according to protrusion, mouth opening (assisted and unassisted) and mediotrusin movements (R&L) at the 7th day post treatment were higher than the values pretreatment and these mean were remained constant at 14th day post treatment so, a highly significant difference P<0.01 in period (1st day pre - 7th day post) and (1st day pre -14th day post) treatments have been stated, while the data outside the comparison limit have been registered in period (7th-14th ) day post treatment. Table (4).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Day</th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
<th>pre -7th post</th>
<th>7th post-14th post</th>
<th>pre -14th post</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTR</td>
<td>protrusion</td>
<td>1st pre</td>
<td>5.44</td>
<td>5.44</td>
<td></td>
<td></td>
<td>0.001 HS</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7th post</td>
<td>7.48</td>
<td>7.48</td>
<td></td>
<td></td>
<td>OCL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14th post</td>
<td>7.50</td>
<td>8.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTR</td>
<td>Mouth opening</td>
<td>1st pre</td>
<td>38.23</td>
<td>8.54</td>
<td></td>
<td></td>
<td>0.001 HS</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td>unassisted</td>
<td>7th post</td>
<td>44.80</td>
<td>4.45</td>
<td></td>
<td></td>
<td>OCL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14th post</td>
<td>44.30</td>
<td>2.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTR</td>
<td>Mouth opening</td>
<td>1st pre</td>
<td>39.54</td>
<td>9.55</td>
<td></td>
<td></td>
<td>0.002 HS</td>
<td>0.002 HS</td>
</tr>
<tr>
<td></td>
<td>assisted</td>
<td>7th post</td>
<td>48.72</td>
<td>4.45</td>
<td></td>
<td></td>
<td>OCL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14th post</td>
<td>48.50</td>
<td>2.42</td>
<td></td>
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</tr>
</tbody>
</table>
Table (4) Summary statistics concerning studied Measurements (Protrusion), (Mouth opening) and (mediorusion) movements in relative to effectiveness DTR therapy:

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st pre</td>
<td>7th post</td>
<td>14th post</td>
<td>1st pre</td>
<td>7th post</td>
<td>14th post</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>6.04</td>
<td>10.00</td>
<td>10.60</td>
<td>6.44</td>
<td>9.60</td>
<td>9.40</td>
</tr>
<tr>
<td></td>
<td>1.37</td>
<td>2.36</td>
<td>2.72</td>
<td>2.69</td>
<td>2.14</td>
<td>2.22</td>
</tr>
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</tr>
</tbody>
</table>

(*) HS: High Significant at P<0.01; OCL: Outside the comparison limit; ; R: Right; L: Left; DTR: Disclosure Time Reduction; SD: standard deviation; 1st pre: first day pretreatment; 7th post: 7 day post-treatment; 14th post: 14th day post treatment.

Discussion

Depend on the fact that the more time taken for excursive movement (more than 0.4 seconds) leads to longer compression of the periodontal ligament, thereby leading to muscle hyperactivity through the biofeedback system and lactic acid build-up with ischemic changes. The lactic acid then accumulates over time as these prolonged excursive contacts continually hyperactivate the involved musculature, thereby producing the ischemic symptomatology often seen in the MPDS subject (8). Disclosure Time Reduction (DTR) is reduced using a T-Scan guided and measured occlusal adjustment procedure known as Immediate Complete Anterior Guidance Development (ICAGD), where in posterior excursive occlusal interferences are removed selectively from all working and balancing molar and premolar contacts, until the Disclosure Time value becomes < 0.5 seconds per excursion (9). The fact that mentioned above was confirmed by current study when the DTR (ICAGD) therapy appeared an amazing effect on sudden improvement of all signs and symptoms associated with MPDS and this agree with (10, 11).

Many authors have employed computerized occlusal analysis in treating muscular pain (12, 13, 14). In a few controlled clinical occlusal adjustment studies the treated subjects showed dramatic improvements in symptoms (15, 16, 17), while other studies using different occlusal adjustment techniques could not find a correlation between their occlusal corrections and muscular pain symptoms (18, 19, 20).

Interestingly, because the mean values remain constant in subsequent measurements from 7th day post treatment and 14th day post treatment and
registered a highly significant difference between the 1st day pretreatment and 14th day post treatment, so these findings of the current study showed that the effectiveness of this type of therapy was appeared from 1st day and remained constant to 14th day and this agree with Prafulla who concluded Chronic painful muscular TMD symptoms, functional restrictions, and the resultant levels of emotional depression from living with chronic painful symptoms, were all dramatically improved within the treatment group within weeks after they underwent ICAGD. Symptom improvements were maintained over the 6-month period of observation (21). Significantly longer disclusion time, higher posterior frictional contacts, and more TMD symptoms were observed in the post-orthodontic group, suggesting that orthodontic treatment increases posterior tooth friction. Computerized occlusal analysis is an objective diagnostic tool determining the quality of excursive movements following orthodontic treatment (22). The ICAGD enameloplasty significantly reduces excursive muscle contractions after completion of the first ICAGD treatment session (23). The selective grinding showed a statistically significant reduction of action potentials recorded by the surface electromyography of temporal muscle (24). Dudhia et al described that functional interference i.e. clicking and hypermobility occur when remodeling of TMJ achieves sufficient extent and bulk also be explained on the basis that MPDS, although originating as a functional problem, ultimately can lead to organic disease of TMJ (degenerative arthritis) or muscle (contracture) (25).

**Conclusion**

The results showed the DTR less than 0.5 seconds reduces musculoskeletal-based symptoms of MPDS patients, and this method can be used clinically with highly success in treatment MPDS.

**Conflict of Interest:** None

**Funding:** Self

**Ethical Clearance:** Not required

**References**


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Estimation of Selenium and Toxic Metals (Mercury, Lead) in Some Type of Canned and Fresh (Meat and Fish)

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Abstract

The present study was aimed to estimate the concentration of selenium Se and toxic metals (mercury Hg and lead Pb) in canned and fresh (meat and fish) from different countries, the results show that the concentrations of Se were above the limits and was ranged between 6.25 ppm to 19.12 ppm in fresh and canned meat and between 0.27 ppm to 9.23 ppm in fresh and canned fish, the concentration of Hg was also above the permitted limits and was ranged between 0.002 ppm to 2.1 ppm in fresh and canned meat and between 0.1 ppm to 3.5 ppm in fresh and canned fish, while the concentration of Pb was within the limits and was ranged between 0.029 ppm to 2.3 ppm in fresh and canned meat and between 0.095 ppm to 1.59 ppm in fresh and canned fish.

Keywords: Fresh meat and fish, Canned meat and fish, Selenium, Heavy metals.

Introduction

The analytical control of heavy metals in food is particularly important, since these pollutants are notably cumulative in nature and, therefore, can be toxic to humans. Their determination in foods of animal origin is, thus, of interest.

Foods contain a wide range of elements such as selenium (Se), sodium (Na), potassium (K), iron (Fe), calcium (Ca), copper (Cu) and zinc (Zn). Many of these metals are essential in living organisms. Metals and other elements can be naturally present in food or can enter food as a result of human activities such as industrial and agricultural processes. Many elements that are present in seafood are essential for human life at low concentrations [¹][²][³].

The metals of particular concern in relation to harmful effects on health are mercury, lead, cadmium, tin and arsenic. Mercury and lead are often referred to as heavy metals [⁴][⁵]. The toxicity of these metals is in part due to the fact that they accumulate in biological tissues, a process known as bioaccumulation. This process of bioaccumulation of metals occurs in all living organisms as a result of exposure to metals in food and the environment, including food animals such as fish and cattle as well as humans.

Toxicological and environmental experts have shown concern for the increasing cases of food contamination with these heavy metals over the years as reported in several literatures [⁶][⁷][⁸][⁹]. Maximum levels for mercury, lead, cadmium and tin in foodstuffs have been set by Commission Regulation No 1881/2006, the framework EU legislation which sets maximum levels for chemical contaminants in foodstuffs.

In the current study the concentration of Se, Hg and Pb were investigated of fresh and canned (meat and fish).
Material and Methods

The study was carried out during 2018. The aim was to identify and quantify the content of Se and heavy metals Hg and Pb) in Twenty-eight type of meat and fish. Apart of sample was fresh and another part was canned from different country.

Apparatus:

All glassware was soaked overnight in 10% (v/v) nitric acid, followed by washing with 10% (v/v) hydrochloric acid, and rinsed with deionized distilled water and dried before using. A Shimadzu Model 12-630-AA Atomic Absorption/flame Emission equipped was used to determine Selenium, Mercury and Lead.

Sample preparation and digestion:

Twenty-eight fresh and cans of fish and meat samples dried in oven at 1050C for 24h, then this sample transferred to desiccators, to remove moisture, then samples leaved to matching with room temperature. The tissue crush by ceramic mortar, then 1g weight from tissue powder and put it in 25 ml glass volumetric flasks and vent closed by a glass plug during digestion. The sample is then taken and digested promptly as follows, the 1gm was weighed into a 25ml glass volumetric flask, and 4.5ml of concentrate HNO3 and 1.5ml of concentrate HClO4 were slowly added, the flask was then shaken well to blend between powder of tissue and acids, the flask was covered by watch glass and left for 24h under the exhaust fan to complete the digestion process. After that, samples were warm at 700C for 2-3h in block digestion, the flasks take out block the digestion, 2-3ml of deionized distilled water was added. Then the opening flasks warmed again in block digestion at 700C until the volume of solution reduced to 2ml. The samples transferred to a flask (50 ml) and complete the volume of deionized distilled water. The solution put in clean plastic tube and centrifuge was used with 3500r/m to 30m, the filtered solution put again in flask (50 ml) and this solution was ready for measurement by atomic absorption spectrophotometer [10].

Results and Discussion

The concentrations of Se, Hg and Pb in fresh and canned (meat and fish) were analyzed to assess the amounts of these metals.

The results indicate that the concentration varied from 6.25 to 19.12 ng/ml (mean = 11.73 ng/ml) for Se in meat samples and from 0.27 to 9.23 ng/ml (mean = 1.94 ng/ml) in fish samples as shown in (Table 1, Figure 1). It is observed (Table 2, Figure 2) that fresh meat, has lower concentration of Se than the canned meat, while the concentration of Se in the fresh fish is about four times that observed for the canned fish.

As well as the results indicate that the concentration varied from 0.002 to 2.1 mg/l for Hg in meat samples and from 0.1 to 3.5 mg/l in fish samples, the limits set by US- EPA for mercury was 0.50 µg/g in fish [6]. Any study doesn’t record mercury exist in meat samples [11]. The results indicate the concentration of Hg in meat and fish samples exceed normal levels (Fig 1). Bioaccumulation of Hg by fish and shellfish in canned food item can be a rich source of metals, and of the serious contamination of foods that occurs from time to time during commercial handling and processing, most countries monitor the levels of toxic elements in foods [12]. Hg has been recognized as severe environmental pollutant, with high toxicity even at low concentrations it has the ability to enter into biological systems [13], it has strong tendency to accumulate in aquatic food chain and about 95% of the methylmercury in humans is originated from the ingested fish [14]. Mercury and methylmercury are neurological toxicants to humans [15]. However, the levels the metals in fresh meat and fish are higher than the canned meat and fish as shown in (Table 2 and Figure 2). The high level of this metal in fresh food may be connected with environmental factors such as polluted soil and polluted waste water used for irrigation of the farms.

Determinate mean concentrations of pb in meat and fish were 0.53 mg/l and 0.5 mg/l respectively (Table 1, Figure 1), this results are higher than acceptable Concentrations of pb limits in fish according to FDA [16], but concentrations in canned meat and fish was below the prohibited limits for this element (Figure 2), which is 0.5 mg/ kg as given by (FDA), while found high concentration of Pb in fresh meat and fish (Table 2). An exposure to (pb) for a long time can increase (pb) in human body and lead to cause many serious diseases
such as anemia, Pale skin, abdominal pain, Nausea, Vomiting and Joints paralysis, exposer to (pb) for a long period may cause kidney filler and reducing fertility and increasing probability of pregnant or Incidence of congenital malformations [17].

Table 1. Total concentration of Metal (Se, Hg and Pb) in various varieties of Meat and Fish.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Se (ng/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
</tr>
<tr>
<td>Fresh and canned Meat</td>
<td>14</td>
<td>6.25-19.12</td>
</tr>
<tr>
<td>Fresh and canned Fish</td>
<td>14</td>
<td>0.27-9.23</td>
</tr>
</tbody>
</table>

Table 2. Concentration of metals (Se, Hg and Pb) in fresh and canned (Meat and Fish).

<table>
<thead>
<tr>
<th>Mean Concentration</th>
<th>Meat</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Canned</td>
</tr>
<tr>
<td>Se (ng/ml)</td>
<td>9.805</td>
<td>12.48</td>
</tr>
<tr>
<td>Hg (mg/l)</td>
<td>1.625</td>
<td>0.39</td>
</tr>
<tr>
<td>Pb (mg/l)</td>
<td>1.25</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Figure 1. Concentration of metals (Se, Hg and Pb) in fresh and canned (Meat and Fish).
Conclusion

In this study, the levels of Se, Hg and nickel and Pb in fresh and canned food samples were investigated. The result of the analysis showed that these heavy metals were present in the selected canned and fresh foods. Considerable differences were found in the levels of these metals among the samples. The level of Se was found within acceptable limit set by World Health Organization (WHO). However, Pb and Cd call for concern as they were found to be above permissible acceptable limit set by World Health Organization (WHO).

The canned samples generally, recorded lower concentration of heavy metals as compared with the fresh fish and meat samples and could be considered safer for consumption. Consumption of fishes grown in rivers and fish ponds within the study area should be avoided in order to prevent Hg or Pb poisoning due to accumulation over time, which could pose a risk to human health due to the accumulation of these elements in the sensitive internal organs such as liver, kidneys, and brain. So we recommend tightening health control methods and ways of breeding fish caught and processed water that equips fish farms and methods of disposal of industrial waste, which has the main reason for the pollution of fish and meat and determine the extent of the safety and validity of these fish and meat for human consumption.

Ethical Clearance: Taken from Chemistry Department of Science College, Basrah university committee.

Source of Funding: Self.

Conflict of Interest: It is nil.

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Profile of Contralateral Patent Processus Vaginalis in Pediatric Patients with Unilateral Inguinal Hernia

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Abstract

Objective: Reporting the incidence of contralateral patent processus vaginalis (CPPV) in pediatric patients with unilateral inguinal hernia. Methods: The study was conducted from November 2010 - February 2012. Patient’s identity were recorded for registration number, name, age, gender, address and date of examination. The surgery was conducted by a trainee pediatric surgeon as a researcher and resident. High ligation procedure and diagnostic of contralateral PPV. Results: This study included 40 pediatric patients of various ages diagnosed with lateral inguinal hernia. There were 31 male patients (77.5%), 24 patients (60%) with right inguinal hernia patients, and 14 patients (35%) aged 0-1 years. Conclusion: The transinguinal laparoscopic contralateral PPV technique is helpful in diagnosing the presence of contralateral PPV. The incidence of contralateral PPV in this study was 27.5%. Furthermore, early age increased the incidence of contralateral PPV.

Keywords: laparoscopic transinguinal technique, Patent Processus Vaginalis, unilateral inguinal hernia,

Introduction

Inguinal hernia surgery is the most frequent procedure performed by pediatric surgeons, and is a frequent case that referral patient consult to pediatric surgeons(1). Most children have unilateral hernia. Only a small proportion have metachronous contralateral hernias(2).

Lateral inguinal hernia in children arises because of the delay or failure of closure of peritonei processus vaginalis known as the Patent Processus Vaginalis (PPV). Abnormalities of the closure of the processus vaginalis can cause various kinds of disorders including lateral inguinal hernia, communicating or non-communicating hydrocele fungiculi, testicular hydrocele, cryptorchidism. The incidence rate of lateral inguinal hernias in infants or children is 1-4.4%, in which it is mostly found in boys than girls, and 10-15% are bilateral(3).

The diagnosis of inguinal hernia in children can be enforced based on clinical conditions, namely anamnesis in the form of a lump in the groin, especially when the patient cries or strains, and disappears when the patient sleeps. Moreover, a silk glove sign appears on physical examination. Examination of the silk glove sign is difficult because it depends on the examiner’s experience. Therefore, this causes a less accurate diagnosis of PPV based on physical examination, especially on the contralateral side(4, 5).

Contralateral hernia in the future will cause higher disadvantages, twice the risk of anaesthetics, and doubled parental anxiety and patient’s stress level. Some investigators undertake routine contralateral exploration to avoid those disadvantages. However, there are several harms reported regarding contralateral exploration, including the risk of testicular injuries and vas deferens, prolonged operative duration and negative exploration(6).

Over the past 60 years, contralateral inguinal exploration procedure in pediatric patients with unilateral lateral inguinal hernia is still under debate. Since 1950, there have been many reports on how should
contralateral inguinal exploration be indicated because of the contralateral PPV. Pediatric surgeons can take many options to treat PPV. First, observing and correcting should clinical symptoms of inguinal hernia appear. Second, a routine contralateral inguinal exploration during unilateral inguinal hernia repair surgery. Third, performing a contralateral exploration only if the PPV is found per laparoscopy during unilateral inguinal hernia surgery. Fourth, only performing selective contralateral exploration in preterm infants, low birthweight and significant anaesthetic risk.

Based on these considerations, the decision to explore contralateral has been preceded by several attempts to diagnose the presence of contralateral PPV. Some of preoperative examinations are intra-operative pneumoperitoneum (Goldstein test), herniography, ultrasonography and laparoscopy. Laparoscopic technique has grown rapidly as a diagnostic tool to look for the presence of contralateral hernia or PPV, while at the same time prevents the second surgery and is a minimally invasive technique.

This study was conducted to explore the benefits of transinguinal laparoscopy in patients undergoing unilateral inguinal hernia surgery by a single surgeon and to identify the presence of a contralateral PPV in children of various ages.

Methods

The population of this study were all patients undergoing lateral inguinal hernia surgery at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from November 2010 to February 2012. The samples of this study were pediatric patients of unilateral inguinal hernia who met the inclusion and exclusion criteria. Inclusion criteria included children ≤ 12 years who suffered from unilateral inguinal hernia and patients who had never had hernia surgery, either left or right. Exclusion criteria included lateral inguinal hernia patients with comorbid diseases such as ascites, hydrocephalus post VP shunt, gastrochisis, sex anomalies, support muscle tissue disorders, incarcerated lateral inguinal hernia patients, and bilateral inguinal hernia patient (duplex).

All unilateral inguinal hernia patients who would undergo high ligation procedure from November 2010 to February 2012 who met the inclusion and exclusion criteria were included in the study. Patient’s identity was recorded including register number, name, age, gender, address and date of examination. The surgery was conducted by a trainee pediatric surgeon as a researcher and resident. High ligation procedures and contralateral PPV diagnostics were carried out in accordance with standard surgery procedures applicable at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The surgical procedure includes when the hernia sac is opened, the probe is attached and tied so that compressed abdominal air does not come out, and a laparoscopy camera is inserted with a 30-degree lens unruly peeking at the PPV counterpart.

The research was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from November 2010 to February 2012. Data were collected from samples and processed as descriptive data. Statistical analysis was carried out using IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA).

Results

This study included 40 pediatric patients of various ages diagnosed with lateral inguinal hernia, with 31 male patients (77.5%), 24 patients (60%) with right inguinal hernia (Figure 1), and 14 patients (35%) aged 0-1 years (Table 1 & 2).
Table 1. Basic research data

<table>
<thead>
<tr>
<th>Basic Data</th>
<th>n (%)</th>
<th>cPPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (77.5)</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (22.5)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Location of Inguinal Hernia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>24 (60)</td>
<td>8 (33.3)</td>
</tr>
<tr>
<td>Left</td>
<td>16 (40)</td>
<td>3 (18.7)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 year</td>
<td>14 (35)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>1-3 year</td>
<td>10 (25)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>3-6 year</td>
<td>10 (25)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>6-12 year</td>
<td>6 (15)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 2. Results Distribution of CPPV Locations by Age Group

<table>
<thead>
<tr>
<th>Age</th>
<th>cPPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>0-1 year</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>1-3 year</td>
<td>3 (30)</td>
</tr>
<tr>
<td>3-6 year</td>
<td>3 (30)</td>
</tr>
<tr>
<td>6-12 year</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

In this study, the highest incidence of lateral inguinal hernia was found in male participants (31; 77.7%), which is in accordance with previous literatures. The incidence of lateral inguinal hernia in men is 4–6 times that of women\(^3\). Other studies stated the ratio of men: women 3: 1 and 10: 1. However, there was no significant gender difference in the group of preterm infants\(^4\).

Participants in this study mostly had right inguinal hernia (60%), which is in accordance with previous studies that approximately 60% of hernias occur on the right side. This condition was found in both men and women due to the slower process of closing the processus vaginal\(^1\).

From age distribution, the incidence of lateral inguinal hernia was mostly found on the age group of 0-1 year (35%), followed by 1-3 year (25%) and 3-6 year (25%). The incidence decreased at over 6 years old (15%). There were 11 cases of contralateral PPV or 27.5% for all ages, contrary to the finding of Rothenberg and Barnet that stated that the incidence of <1 year contralateral hernia was 100% and 68.5% for ages >1 year\(^2\). However, the finding of this study is closer to the results of previous studies that pointed out 14–41% incidence of contralateral hernias\(^21\). Unlike previous studies that only mentioned the incidence of contralateral
hernias at 11%, this study had a nearly double higher result(2, 4, 10).

The graph above showed that contralateral PPV incidence mostly occurred in cases of right lateral inguinal hernia (20%), while the left side was only 7.5%. A study conducted by Surana and Puri found that the incidence rate of contralateral hernia was 10.3% in infants aged 1 week to 6 months, while patients with left hernia who developed right hernia was 16.6%(10, 22). Meanwhile, previous study conducted for 20 years found that 41% of patients with left inguinal hernia would experience future right inguinal hernia, whereas only 14% of patients with right inguinal hernia would develop left inguinal hernia(21). Compared with the two studies, this study found that the incidence of contralateral PPV in cases of right hernia was higher. This finding could become a consideration that in cases of unilateral lateral inguinal hernia, diagnostics must first be performed prior conducting contralateral exploration(23).

This study found that transinguinal contralateral laparoscopy was very helpful in diagnosing contralateral abnormalities. Therefore, appropriate action could be taken at the time of the first surgery, as well as knowing the certainty of contralateral PPV to reduce morbidity due to negative contralateral exploration. The disadvantages of this surgery require more expensive costs and a relatively longer time.

Conclusions

The transinguinal technique of contralateral PPV is very helpful in diagnosing contralateral abnormalities. Therefore, appropriate action could be taken at the time of the first surgery, as well as knowing the certainty of contralateral PPV to reduce morbidity due to negative contralateral exploration. The disadvantages of this surgery require more expensive costs and a relatively longer time.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Funding: None

References

Medicolegal Analysis of Sodomy Cases at Beni-Suef Governorate From January 2011 to December 2015 – A Retrospective Study

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Abstract

Sodomy is anal intercourse between 2 men (homosexual sodomy) or a man and a woman (heterosexual sodomy) also known as buggery, when the passive agent is a child, sodomy is referred to as paederasty. This study sheds some light on one sensitive Egyptian issue, sodomy cases, with quantification of the demographics and medico legal findings, related to female child and male cases presenting to Beni Suef governorate Forensic Medical Authority, Egypt, between 2011 and 2015. 51 cases of sodomy were analysed, the mean age of victims is 9.79 years in children and 24.30 years in adults. 74.19% of cases were male children while 85% were adult females. Cases occurred mainly in urban areas for both child (61.29%) and adult (55%) victims. Frequency of sodomy cases shows an ascending manner. Most child victims had primary education 77.42%, unlike 35% high education in adult victims. 96.67% of assailants had no relation to children, 50% were relative in adult cases. Multiple assailants were present in 13% of child victims, 30% in adult victims. Physical force was the commonest in child 51.62% and adult 50% victims. assailants were guilty in 54.84% of child victims, 50% in adult victims. Forensic examination of children took (2-5 days) in 61.30%of child cases and (1-2 weeks) in 50% adult cases. Semen was detected in 19.35% of child cases and in 15% of adult cases. Genital Injuries were found in 45.16% of children and55% in adult cases. Rape accompanied sodomy in 19.35% of child cases and 25% of adult cases. Conclusion—these analyses demonstrate that the very low incidence may also suggest gross underreporting; making awareness of importance of reporting of sexual offences is mandatory.

Key words: Sodomy; Assailants; Forensic examination; Physical force; Genital Injuries

Introduction

Sexual assault (SA) is any sexual behavior or contact occurs without a distinct consent from the recipient or any exposure to an inappropriate sexual content (¹). Sexual abuse, affecting children and women of all ages and socio-economic levels, is considered a major human rights violation and a worldwide problem (²³). Sodomy is anal intercourse between 2 men (homosexual sodomy) or a man and a woman (heterosexual sodomy) also known as buggery, when the passive agent is a child, sodomy is referred to as paederasty (⁴). Age of consent for sexual intercourse in Egypt is 18 year and sodomy is illegal (⁵). The legislation in Egypt makes no straightforward mention of homosexuality, they frequently use other laws to criminalizes the act and punish the perpetrator (⁶). Sexual violence and especially sodomy in Arab-Muslim societies depend mainly on the cultural and religious norms; which is a neglected area of research (⁷). Egypt’s Forensic Medical Authority

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affiliated to the Ministry of Justice that is responsible to provides a medico-legal investigations and reports, divided into two parts in Beni Suef; Beni Suef morgue, clinic and Laboratory which cover Beni Suef, Ehnasia, Beba, Elfashn, Somosta, Naser & Elwasta provinces.

This study sheds some light on sodomy cases, with quantification of the demographics and medico legal findings, related to child and female cases presenting to Forensic Medical Authority between 2011 and 2015.

**Materials and Methods**

This is a retrospective study of all alleged sodomy cases in Beni-Suef province from the start of 2011 till the end of 2015.

**Data collection**

The data was collected from archived reports of medico legal authority registered in Beni-Suef province. Data was collected from all reports contain claims of sodomy cases in a collection sheet based on available information of medico-legal reports. The study included closed claims only since the claims that were not yet closed were not available. The claims ‘files included the statement of the claim, medical reports, experts ‘opinions and the decision of the court.

A total number of 51 registered sodomy cases were studied according to police forwarding letter and Medical officers form which included all the relevant information such: Date, Case result, Victim mentality, Age, Residence, place of the assault, Relationship of assailants to the victim, Time between the sexual assault and forensic examination, Educational status of the victims, Internal examination, External examination and injuries, Number of assailants, Type of sexual assault , Method to overcome the resistance of victim, Presence of semen.

**Ethical considerations**

Number Ethical concerns need to be addressed, due sensitive study nature especially in our country. No identifiable personal information on the Victims, witnesses, or the suspect was included in the coding or analysis. This study was performed according to guide lines of Ethical Committee at Beni-Suef University, Egypt.

**Statistical Analysis**

The data was coded and entered using the statistical package for Social Sciences SPSS version 21. The data were summarized using a descriptive frequency and percentage for quantitative values, relation between data grouped was tested by Chi Square test for quantitative variables. Statistical differences (P-values) less than or equal to 0.05 were considered statistically significant.

**Results**

A total number of 51 registered sodomy cases were analysed, the mean age of child victims is 9.79 years while the mean age of adult victims is 24.30 years. Amongst sodomy cases, we found that male children were more exposed to sodomy (74.19%) than female children (25.81%), while adult females go to court (85%) more than adult males (15%). The relation between gender and age of victims was highly significant (p <0.001, Table1) The prevalence of sodomy cases in urban areas in the governorate was more than rural areas for both child (61.29%) and adult (55%) victims with no statistical significance (p =0.656, Table1) Frequency of sodomy cases shows an ascending manner over the 5 years. On plotting years and age of victims, 2015 has the highest percent of child Cases (32.26%) while 2015 and 2014 has the highest percent of adult Cases (30%) The relation between victim number and age over years was not significant (p= 0.607, Table1) Most child victims had primary education (77.42%), in comparison to high education in most adult victims (35%). That relation was highly statistically significant (p <0.001, Table1) All child victims show average mentality (100%), while (10%) of adult victims were mentally retarded with no statistical significance (p =0.072, Table1)

The majority of assailants had no relation to child victims (96.67%), unlike (50%) of relative assailant in cases of adult victims. That relation was highly statistically significant (p <0.001, Table2) Multiple assailants were present in child victim cases (16.13%) and adult victim cases (30%) also in the least percentage with no statistical significance (p =0.240, Table2). 61.29% of child assaults were at unknown place, unlike 50% of adult cases that occurred at victim residence. That relation was highly statistically significant (p <0.001, Table2) Physical force was the commonest type of force used in child (51.62%) and adult (50%)
victims, while weapons used only in (16.13%) of child victims and in (20%) of adult victims. This relation was found none statistically significant (p =0.758, Table2). (54.84%) of assailants in case of child victims were found guilty, while in cases of adult victims, 50% of assailants were also found guilty. That relation has no statistical significance (p =0.735, Table2).

The majority of forensic examination of took place few days (2-5 days) after assault in (61.30%) of child cases, while in adult cases (50%), it took few weeks (1-2 weeks). That relation was statistically significant (p =0.016, Table3) Forensic examination can detect semen in (19.35%) of child cases and in (15%) of adult cases. That relation was statistically non-significant (p =0.690, Table3) Genital Injuries with a skin tear were the commonest type of injuries in both; child (45.16%) and adult (55%) cases, while no injuries were found in of child (32.26%) and adult (15%) cases. That relation has no statistical significance (p =0.585, Table3). Rape accompany sodomy in (19.35%) of child cases and (25%) of adult cases. That relation was statistically non-significant (p =0.632, Table3).

**Discussion**

This is a retrospective study of all alleged sodomy cases in Beni-Suef province from the start of 2011 till the end of 2015. The data was collected from archived reports of medico legal authority registered in Beni-Suef province. A total number of 51 registered sodomy cases were analysed, this number is most probably due to under reporting of cases; making determination of accurate incidence numbers not possible (8). Under reporting cases including younger and cognitively delayed children suffering severe and repeated abuses within the family (9), 83% of young adults experiencing sexual assault won’t reach the police (10).

This study revealed that, the mean age of child victims is 9.79 years while the mean age of adult victims is 24.30 years, consistent results were stated by (Peterson; Melkman et al.; Office for national statistics) (9,10,11). These findings can be explained by underreporting to police and health authorities by the older survivors who may fear loss of societal respect (12). Older children have the language, attention and cognitive skills necessary to provide clear and detailed evidence of suspected abuse (13).

Amongst sodomy cases, we found that male children were more exposed to sodomy (74.19%) than female children (25.81%), Kloppen et al. (14) findings are different as between 6% and 30% of girls and 1–12% of boys in the Nordic countries are estimated to fall victims to contact child sexual abuse. These differences may be due to the nature of sodomy. In our study adult females go to court (85%) more than adult males (15%).

Regarding gender, studies generally indicate that in cases of sexual abuse, male victims are more reluctant to disclose events of abuse, are less consistent in their accounts, and that their testimonies are less likely to be viewed as credible by investigators (9,15,16).

The prevalence of sodomy cases in urban areas in the province was more than rural areas for both child (61.29%) and adult (55%) victims. According to WHO (17) some people do not trust police, especially in rural areas where other authorities are not accessible.

Frequency of sodomy cases shows an ascending manner over the 5 years. On plotting years and age of victims, 2015 has the highest percent of child Cases (32.26%) while 2015 and 2014 has the highest percent of adult Cases (30%). These results are consistent with (10). That increase may be attributed to the improvement in reporting after the Egyptian revolution of 2011.

Most child victims had primary education (77.42%), in comparison to high education in most adult victims (35%). Our results are consistent with Patterson & Campbell (18). Lippert et al. (19) explained as, preschoolers are less likely to disclose abuse, both initially as well as in the forensic interview and if they do disclose, their testimony tend to be shorter and less detailed than testimony from older children. On the other hand high education usually increases awareness about the importance of abuse reporting.

All child victims show average mentality (100%), while (10%) of adult victims were mentally retarded. Similar findings were mentioned and explained by Ghatti et al.; Connolly et al.; Office for national statistics (10,16,20) as Cognitive delays exert effects on children’s ability to provide informative evidence and hence on the ability to assess their veracity, though existing findings are mixed. Assessments of their credibility concerning allegations of abuse are made with less certainty.
The majority of assailants had no relation to child victims (96.67%), unlike (50%) of relative assailant in cases of adult victims. Roye et al.; Melkman et al. (9,21) agree with our results, the convenient explanations could be of Rizzo et al. (22) that the database was formed by forensic examiners after filling a charge in police station by the legal guardian of the child that is considered a family scandal especially if the offender is a family member. Female victims of physical intimate partner violence are more likely to report anal intercourse according to Roye et al. (21).

Multiple assailants were present in child victim cases (16.13%) and adult victim cases (30%) also in the least percentage. The findings of Melkman et al.; Office for national statistics (9,10) are in agreement with our results. The possible explanation of our results could be due to threats of violence or death which has been found in this study (12).

Physical force was the commonest type of force used in child (51.62%) and adult (50%) victims, while weapons used only in (16.13%) of child victims and in (20%) of adult victims. Similar results were reported by Cross et al.; Whitaker et al.; Office for national statistics (10,15,23). Disclosure may be easier for the child traumatized by an aggressive, one time sexual act. Existing evidence suggests higher rates of affirmation concerning reports of more severe abuse (15).

61.29% of child assaults were at unknown place, unlike 50% of adult cases that occurred at victim residence. Office for national statistics (10) reported similar results. The possibility of being caught in the act is quite slim especially when the assailant majority had no relation to child victims (12). Unlike 50% adult victims with relation to the assailant; there is increasing evidence that anal intercourse is more common among female victims of physical and/or sexual intimate partner violence (24).

Nearly 50% of assailants in case of sodomy victims; children and adults were found guilty. Consistent results reported by Priebe and Svedin; Melkman et al. (9,25). The explanation stated by Talwar & Crossman (27) were the most likely, as, elementary school children’s developing cognitive skills are also related with a greater capacity for telling and maintaining lies. Priebe and Svedin & Meyers (25,26) have established that timely delayed disclosure, greatly decreases the likelihood that the case will be reported, investigated and that charges will be filed and the abuser most likely won’t be convicted.

The majority of forensic examination of took place few days (2-5 days) after assault in (61.30%) of child cases, while in adult cases (50%), it took few weeks (1-2 weeks). Forensic examination can detect semen in (19.35%) of child cases and in (15%) of adult cases. Akinlusi et al.; Rizzo et al. (11,22) reported similar results and explain this delay by the longer duration to disclosure. Children particularly believe assailants’ threats and would not report until parents discover. The fear of stigmatization could be responsible for delayed disclosure in adult. Individual differences at the level of forensic examiners (e.g., years of experience or attitudes) may explain considerable variance in their assessments (9).

Genital Injuries with a skin tear were the commonest type of injuries in both; child (45.16%) and adult (55%) cases, while no injuries were found in of child (32.26%) and adult (15%) cases. These findings are consistent with the study of Office for national statistics; Rizzo et al. (10,22). Appropriate explanations are that; older girls possibly try to physically defend themselves and the bodily lesions could be the results of a physical opposition, whereas children don’t attempt to fight back. In addition, less invasive acts as touching and fondling are known to occur much more frequently than penetration in cases of younger children (28). Moreover, the delay in seeking medical examination may also explain the absence of evident genital or bodily lesions (29). It is possible that in less severe events, common motivations underlying intentional fabrication of accounts of abuse, such as taking revenge at a third party, a cry for attention or help, or concealing consented sexual activity with the accused or another for fear of parental retribution (30).

Rape accompany sodomy in (19.35%) of child cases and (25%) of adult cases. Office for national statistics (10), reported similar results. Past research has shown that anal intercourse is associated with intimate partner violence victimization among women. Female victims of physical intimate partner violence are more likely to report anal intercourse. (21,24)
Limitations

This study has a limitation; the Number of cases was relatively small; the very low incidence may also suggest gross underreporting. Our analyses include only the referred cases to Forensic Medical Authority by law enforcement accompanied by family members or on their own. Sexual assault cases, like sodomy considered a shame especially in Upper Egypt governorates and can be scandalous if the offender is a relative. In case of child abuse many guardians refuse to go to police, so these cases are missed out. Despite that limitation, we report important results supported by data collected by law enforcement. A joint forensic physician and nurse team performed all the examinations in order to secure objective information. Our results can be used as effect-size estimates to power future clinical prospective studies in hospital abuse cases.

Conclusions

The standard of clinical management of sexual violence involves documentation and treatment of injury, getting forensic materials, detecting prior pregnancy, screening for sexually transmitted infections including HIV and provision of adequate contraception, post exposure prophylaxis and supportive psychosocial counselling. National awareness about sexual offences reporting importance to public and about the standard of clinical management of sexual violence among health care providers is a must.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest : Nil

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Genetic Modification of Mitochondrial DNA in Cancer Cells

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Abstract

Mitochondria is one of the most energy source in the cells, in addition to DNA encoded to several genes which relatively associated with several disease, researchers proved the role of mitochondrial in energy demand to tumor cells in addition to mitochondrial DNA role in cancer initiation and development. The present review explained different objectives related with the mitochondrial role in tumor incidence ; these included Tumor cells energy demands, Mitochondrial Mutations redundancy and allocation, Genetic disparate natural Selection in Tumor initiation, Mitochondrial Modifications Clinical Translations in Cancer, Association Mitochondria, epigenetics and Cancer and finally Injury of Mitochondria in Cancer. The present review concluded that the most important role of mitochondrial as cells an organelles for energy suppliers to tumor cells metastasis and immorality also the mutations of mtDNA and their role in carcinogenesis were well proved in different cancer types.

Key words: mtDNA, cancer, mutation, role mtDNA, energy source.

Introduction

Mitochondria is one of the most important organelles in the cell, the biogenesis and roles are regulated by nucleus by a stable bidirectional crosstalk, mitochondrial DNA (mtDNA) encoded 1% of mitochondrial proteins, while the others encoded by nuclear genomic including the replication and transcription proteins [1]. The mitochondrial genome in human is circular double strand consist of 16.6 kb encoded two ribosomal RNA (12S and 16S), 22 tRNA and 13 essential subunit of protein associated oxidative phosphorylation system (OXPHOS) , Other proteins involved in The electron transport chain (ETC) and the pathway of primary metabolic generating energy carrying molecule ATP which formed of five protein complexes (I-V), involving the complex II is exclusively coded via nuclear genome [2].

The nuclear genome encoded to all proteins subunit that formed the rspiratory systems included 7 subunits of the enzyme in Com I, one subunit of the ComIII, 3 subunits of Com IV and 2 subunits of ComV. As formerly indicated, the mitochondrial proteins, included the mtDNA processing like transcription and translation proteins and replication, encoded by the nuclear genes and are subjected to the mitochondrion by particular transport systems, about 2000 small non-coding RNAs (mitosRNAs) were detected, that have role the natural mitochondrial gene expression control, showed an underestimated scale of mitochondrial functional complexity [3].

Otherwise, the investigations deal with antisense anti-termination tRNAs and delRNAs focus a de nova facts on incoming processing extending the coding prospect of mitochondrial genome [4, 5]. The ETC byproducts of the steadily create reactive oxygen species (ROS) which may seriously harm the mitDNA that cusses different types of mutation and the these lesions accumulation in the mtDNA molecules result in subsequent the dysfunction in mitochondria , like number changes, morphology and functioning, as
noticed in tumor cells \[^6\].

The mitochondrial DNA is more exposed to mutated than nuclear DNA, because of the absence specific proteins like histones and the loss of structure chromatin, rarity of introns, also the less effective recover mechanisms of mtDNA and a direct exposed to the damaging ROS created pending ATP generation within the mitochondria \[^7\].

In spite of low scales of ROS to organize the signaling in the cells and are substantial for normal endurance and reproduction of cells, thumbing ROS generation is repeatedly noticed in neoplastic cells. The theory of mitochondrial free radical of aging the cumulating of harmful mtDNA mutations, inhibition of oxidative phosphorylation, furthermore the disturbance in antioxidant enzymes expression leads to exponential overproduction of reactive oxygen species, This evaded situation consist vicious cycle which is the fundamental of a broad extent of pathologies, phrased as free radical diseases like tumor, atherosclerosis, neurodegeneration, chronic inflammation and diabetes mellitus \[^8\].

Substantially, alongside the apparent creation of oxidative nucleotide deterioration to the mtDNA, the free radicals triggered cancers by different ways, involving settlement of hypoxia inducible factor (HIF)-α, raised the flux of calcium, inhibition of key phosphatases \[^9–11\].

Tumor cells energy demands

Formidable proof now proposes that the energy demands of fast reproducing tumor cells and primary cells are supported by the glycolytic metabolism and the cell differentiation with high energy requested maximize the utilization of mitochondrial respiration \[^12\]. Additionally, the irregular division of stem cells and cancer stem cells have high reproductive potency preferentially utilize glycolysis \[^13\].

Nonetheless, several cancer cell lines possess so high respiration averages, and raising proof supposes a convoluted relation between the energy metabolism of tumor cells with the tumor formation and progression \[^12–15\].

The Motivating metastatic tumor cells without mtDNA (r0 cells) cannot used their mitochondria for respiration develop in culture medium affixed with pyruvate and uridine, though their development is usually extremely slower than the growth of parental cells. These cells display raised cell surface oxygen consuming through their plasma membrane electron transport, a compensative passageway intellect to correct for the setting up of intracellular reductants in respiration obscurity \[^16\], an agent that requires to be taken into consideration in estimating glycolysis participation to total energy metabolism.

Mitochondrial Mutations redundancy and allocation

The difficulty in mutation detection of cancer mtDNA related to small differences linked with the organelles genome. Most significantly, the vicinity of ROS created through normal metabolic events elevated the danger of mtDNA disturbance and insecurity \[^17\].

The impact of ROS in the modifications of mtDNA is upheld via common raise in somewhat cancers of transitions at purines \[^18\], this deterioration, associated with low repair mechanisms comparative to the genome of nucleus, cause an mtDNA mutation range greater than the nuclear genome \[^19\].

Empirical proof supposes that the operation of endogenous mutational are more effect in the range of mtDNA mutation, as dissenting to the exogenous carcinogens such as environment chemicals and UV light \[^20\]. Two additional features are pertinent to explicate the mtDNA disparate redundancy and allocate.

At first, in the cell the mitochondria and their genomes are found in high copy numbers hundreds or thousands. Secondly, the mtDNA is matrilineal heredity in humans, thus there was single mtDNA haplotype but it shows Heteroplasmic pattern due to the impacts of numerous copies). The mutation of germline mtDNA was inherited from mother and are constitutively existed over the offspring. Moreover The Germline mtDNA disparate are valuable for defining individuals to particular haplogroups \[^21\], that able to thereafter be correlated to ancestral matrilineal relations \[^22\].

The haplogrouping can be utilized in relations characteristic among individuals and populations, several haplogroups consist of sequences pattern may be found participates to cancer tendency \[^23–24\], some mutation
in somatic cell manifest haplogroup transmutation \[25\], some types of mutation in mtDNA reflect specific pattern in cancer cells.

Many cancer mutation \[20, 26\] observed specific pattern of mutation landscape in mtDNA related to tumor. Though this latter tendency different to presented in the nuclear DNA, it does make germline patterns shaping primate mutation of mtDNA, mtDNA of human in general includes mutation hotspots in both rRNA and genes coding for protein synthesis, expressing synonymous, non-synonymous, and non-expressed sites, with minimal changes in tRNAs \[27\]. Whole-genome analysis of somatic mutations related to cancer reflects this relative frequency of mutation in divers kinds of sequence \[28, 29\].

The mutation in nuclear and mitochondrial DNA of cancer types are observed as heterogeneous \[20, 26\]. Moreover, the somatic mutations relative rate varying in individual cases was 13 - 63% according to types of cancer and disparate the mtDNA may be found throughout cancer types or exist only in single kind of tumor \[30, 31\].

Lee et al, reviewed a differences between allocate and kinds of somatic mtDNA mutations \[32\]; they clarified express the patterns arising as pertinent throughout tumor kinds. Some genes of mitochondrial have reported somatic mutations that may engaged in tumor initiation. Somatic mtDNA changes throughout cancer types, are riches with non-synonymous disparate compared to synonymous disparate \[29, 30\].

The mtDNA genes coding proteins related to the different complexes of the mitochondrial respiratory chain. The Com I called NADH dehydrogenase is encoded by 7 mtDNA genes \(ND1-6\), including \(ND4L\), its most repeatedly includes disparate correlated to tumorigenesis \(33\). Like \(ND5\) is hardened for somatic mutations \[28, 30, 34\], that may modify development of tumor \[35\].

The Complex III, only \(CYTB\) gene is encoded by mtDNA, includes lower authenticated somatic disparate. the bladder cancer is exception, where this complex is importantly more affected than the others \[36\] and deletion seven amino acid observed in populations that linked with bladder tumor progression in experimental work \[37, 38\]. The Com. IV cytochrome c oxidase have 3 genes expressed in mitochondria \(COXI-3\), researchers found that the mutations in \(COXI\) that linked to colorectal tumor may decrease expression or reduce the respiratory chain effects \[39\].

The Com V or ATP synthase consist of double genes located on mitochondrial DNA \(ATP6 and ATP8\). The \(ATP6\) gene observed to possess more predisposition to mutation than \(ATP8\) in breast cancer patient, which may exhibits alterations in energy metabolism among tumor cells \[40\]. Throughout genes coding proteins, modifications to Complexes I and IV found to be the most effective in stimulating tumor initiation \[41\].

The genome of mitochondria involves 22 tRNAs, constituting small ratio attribution of the nucleotide sequence. The Somatic mutations in tRNA are not redundantly authenticated in correlation to tumor in human, in spite of they are generally engaged in a various other defect in primary respiratory chain \[42\]. As a result of low ration in tRNA mutations that correlated with tumor, they symbolize to show changes to secondary structures \[26\] also the disparate may lead to instability and modified mitochondrial labor \[28\].

In similar manner, the two rRNA genes mutated expressed via mitochondria have high relatively harmful impacts than alterations to genes coding protein \[42\]; though, alterations to the rRNA were low happened. While the genic sites of the mitochondrial DNA are a sensible goal for appreciating tumor mutations, hyper changeable (HV) regions in the non-expressed mtDNA also express common mutational hotspots. the somatic and germline mutations in the mtDNA happened preferentially in two sites of this site, HV1 and HV2 \[43\].

The cancer Researches interested massively on the genetic disparate in D loop, its long segment of control region. Also the non-coding region observed in several animal mtDNAs and is formed by insertion third linear Strand DNA, Given the linkage between control region and the mitochondrial molecular processing, the D-loop mutation can effect in mitochondrial copy number and organization \[44\].

In cancer the mutation in D-loop are well-studied to all mtDNA cancer disparate. This site has large alterations rang to appreciate cell lineages progression
and reproduction [45]. In cancer the D-loop Mutations are also more exist [46], and large scale of somatic D-loop mutations linked to poor prognosis in breast cancer [47].

In spite of possible engagements of D-loop mutations in function of mitochondria in cancer, it is unclear whether these disparate are a causal or simply correlated phenomenon [45, 47, 48].

Genetic disparate natural Selection in Tumor initiation

The theory of cancer initiation is focused on the genetic disorder inn some genes called [49]. Later accumulation of mutation in the genome as a consequence of these disparate in oncogenes, resulted to scan of features identifying tumor progression [50].

The theory of somatic mutation, is hard to reconcile to authenticated mtDNA disparate linked to tumor [51]. Raising proof engaging the tumor risk of mitochondria, initiation, and development contributed to a growing information’s of cancer as a mitochondrial metabolic disease. This phenomena constructed from different suggestions including the natural selection, heteroplasmy, and the combined impacts of genetic modifications throughout the DNA. After the initiation mitochondrial mutations, disparate are subsequently submitted to various molecular, cellular, and population level operations [52].

The carcinogenic intuition via evolutionary operations, the mutations in the genome initiate, then submitted to the natural selection and/or genetic divergence. The impacts of choosing are commonly separated into two groups in tumor investigation: 1st clarifying the negative selection, mean that the harmful alleles are deleted from the population, 2ed the positive selection, mean the beneficial alleles raise in redundancy in the population (may be toward fixation). the mutations may be neutral and not pass to selective forces but stayed reproduce stochastically by drift. Many datasets and applications have been applied to screen for mtDNA mutations selection linked with cancer. In human history, the germline mtDNA mutations consider as a negative selection [53].

According to large samples number of mtDNA suggested that the mutations are submitted to same selective features, in any case of the initiate in normal or cancer cells [54]. The harmful mutations head to be choose against [55], in some healthy tissues alternately express positive selection on mtDNA somatic mutations. a positive selection in liver eliminated mitochondrial labor to reduce damage ensuing from byproducts of metabolism [56].

The mutations selection associated to cancer initiation, reflected of somatic mtDNA mutations through oncocytic cancer types reflects that disparate linked with cancer are indiscernible from random [57]. Meanwhile, a linkage has been found between the number of somatic mtDNA mutations and the patients endurance in breast cancer, with proof for both positive and deliberate negative selection for somatic missense mutations [28].

The mtDNA Scanning studies using metastases in bone represented statistically high variation than metastases in soft tissue and primary [58]. These studies, though apparently paradoxically, accumulatively focus the light on two points in the impact on mtDNA mutations in cancer: the 1st one, the time grade and discrimination of somatic from germline mutations matters, and 2ed, the manners of selection

Mitochondrial Modifications Clinical Translations in Cancer

While the association between the mutation in germline mtDNA and cancer hazard is well discussed overhead (section Mitochondrial Mutations redundancy and allocate), also studies found that mtDNA disparate can apprise cancer disclosure, curing, and prognosis. Due to the presence of mitochondria in large copy number within the cells and are clonal by nature, the have capability to assist in disclosure and detection of some cancer kinds [59].

The usage of mtDNA as a genetic marker not bordered to cancer, yet, as mtDNA disparate correlated to tumor also can be revealed in lower invasive bodily fluids [60], like the usage of urine to diagnosis the cancer in bladder [36]. Also involve the utilization of serum and aspirate fluids for colorectal and breast cancer, diagnosis respectively [61, 62].

Multiple researchers have observed the interest of
mitochondria utilization to cure the cancer\cite{17, 63, 64, 65–67}, even declaring that “comprehension the mechanisms of mitochondrial labor throughout tumorgenesis will be crucial for the next descent of cancer remedies”\cite{68}. According to apoptosis is a major participator in lowering tumor cells in addition to cell death restraint is mitochondrial organized, it logically pursues that the mutations in mtDNA may modify restrains to cancer remedy\cite{69}.

Actually, the case study observed that the mutation in ND4 in ovarian cancer may be causes resistance to chemotherapy\cite{70} also mutations in D-loop have been found that related to chemo resistance in patients with colorectal cancer\cite{71}. Finally the reduction in mtDNA components is related with prognosis enhancement in breast cancer patients subjected to anthracycline-based chemotherapy\cite{72}.

Absolutely, the contrast stand claims for mtDNA gene expression and mutations have confined clinical features, as in ovarian cancer\cite{73}. The association of individualized metabolic operations with myriad diversity in the mtDNA genome and nuclear persuades more work into the used of personalized medicine in curing cancer\cite{74}.

Association Mitochondria, epigenetics and Cancer

The modern cancer researches focused on the mitoepigenetics that mean the epigenetic organization like alterations of mtDNA gene expression and the symmetric interactions with the nuclear genome\cite{75}. The variation in mitochondrial methylation was observed among natural human tissues\cite{76}. Furthermore, allover-genome methylation is correlated with various human diseases, of which the cancer disease\cite{77}. These basic features resulted to an expectation that mtDNA epigenetic assortments able to enhance development of tumor. The impact of mitochondrial give a global manners of gene expression\cite{78} adds credence to a possible linkage between mtDNA copy number and methylation\cite{75}. Furthermore, a study observed a mechanism for this association a mitochondrial disrupt checkpoint may stimulate to remedy injured mitochondria, the mitocheckpoint could prospect change epi-genetic manners and genomic stability when the signaling happens between the nucleus and mitochondria\cite{79}.

These proofs accumulatively suppose the relation of mtDNA epigenetics to cancer, have several benefit formerly evidenced the usage of mtDNA methylation as a biomarker for diagnostic objectives\cite{75, 80}. Yet, potential relations and implementation have yet to be quite explored, particularly when we have shortage in the methylation quantity throughout tumor kinds and stages\cite{81}. Filling these hiatus in acknowledgement may help in bearing down preceding discordant proof among cancer kinds.

Injury of Mitochondria in Cancer

In spite of mitochondrial injury was assumed by Warburg to be an occasion of tumors and this judgment is remain boosted by several investigations, massive proof directs to complexes nuclear genetic modification, some of the mitochondrial labor, being the main cause of cancer with environmentally commanded epigenetic alterations participating in methods that still to be completely understood.

The mtDNA modification happen in most tumors because of the elevation mutation ratio compared with DNA in nuclear and indigent repair mechanics, the involvement mtDNA mutation in the cancer progression has been resides in only a plain number of cancers\cite{82–84}. Another injury in mitochondria due to the oxidative deterioration generated from sustained inefficient respiration and oxygen radical creation, not directly correlated to genome injury may also happen, but how this deterioration plays out in terms of cancer formation and development is still unclear.

Though, the little alterations that participate to rebalancing the energy metabolism toward glycolysis may do a role in tumor inception and development. Many articles authenticate a function of mtDNA mutations in formation of cancer and metastasis. As example, HeLar0 cells which do not form tumors after xenotransplantation were altered into malignant cells able of carcinoma inception by informing mtDNA with particular mutations\cite{85}.

The mtDNA mutation was also presented to be significant for the these cell metastatic tendency\cite{86}. Interestingly, Ishikawa and colleagues reciprocated around the mtDNA between metastatic and non-metastatic cells in breast cancer, after which the original
non metastatic cells initiated metastatic tumors and vice versa [87]. This robustly refers that several mutations in mtDNA, in addition to nuclear mutations, can be significant for metastasis.

**Conclusion**

The present review focused on the association between genius of mtDNA mutations and tumor throughout numerous cancer types. Some Common manner of mutation are explanation by evolutionary operations impacting the existence of these mutations, supplying a significant basis for mtDNA and cancer studies.

Present study recommended using best practices in estimating mtDNA modifications linked with tumor and suppose promising fields for a new research. Modifications to mitochondrial dynamics through tumorigenesis manage the total gamut of possibilities, metabolism, impacting biogenesis and virtually all other quarters of mitochondrial role.

to data concerning the somatic mutation in mtDNA correlated with tumor cumulate, it is clear that modification in the mtDNA contributed in tumorigenesis trigger while others simple fund with cancer develops. Mutations in nuclear-encoded genes, along with corresponding alterations to the cellular labor of ambient cells, could enhance additional mtDNA modifications or applies qualification to what alterations may reproduce.

Defining particular mutations valuable as genetic marker, for this reason, will crave wider sampling of mitochondrial genomes from varied tumor kinds at numerous phases of development, and cautious resolving to estimate the redundancy to mutation in tumor. Mitochondria related with normal and cancer cells show a microscopic tale of two cities. Mutation observed in each genomes, but different were be observed of cellular and molecular powers in the destiny of such disparate. This duality applies the chance to mark both basic acknowledgement of cellular labor and translational medicine.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Nil for review article.

**References**


nature12477.


Influence of Melatonin in the Treatment of Experimental Enterobius Vermicularis Infection

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Abstract

This study aims to realization the conceivable therapeutic of melatonin effects experimental against Enterobius vermicularis in rats. Implement this experiment during the period from August 2019 to January 2020. E.vermicularis infected with male wistar rats orally with dose 15mg/kg melatonin former of body weight for 30 day showed significantly reduction in the number of eggs and worms compared with rats orally with dose 15mg/kg melatonin accompanying and untreated rats for 30 day (P < 0.05).Histologically in intestine examined show increase numbers of leucocytes produce, necrosis significant scatter and reduction this parasite of tissue in rats treated with melatonin. This results show influence of melatonin in the control on Enterobiosis and suggestion that this drug usefulness in Enterobius vermicularis infection therapy.

Keyword: Eggs, Worms, Enterobius vermicularis, Melatonin, Former.

Introduction

Enterobius vermicularis is helminthes more common human parasitic Nematoda infected the bowel but the children worldwide may reach to 40 million infestations in USA and Europe especially school students (¹). Infection may be associated with poor hygiene or behavioral environments in family overcrowded and orphanages where transfer the eggs pinworm from person to another by finger polluted or via anus into mouth directly may transmit by eat contaminated food indirectly (²). The clinical symptoms occurs because the migration of the gravid female worst at night when lays eggs lead to excitement, lack sleep, appetite and weight decrease, vomiting and abdominal pain (³).

There are many drugs can be help in eliminated on pinworm else will not be beneficial, most common drug is mebendazole family these killed the adult worms only addition to increased resistance these drugs wherefore need for the development of new methods for control and enucleate of the parasitic disease (⁴).

Recently studies suggests that melatonin immune enhance function through presence of melatonin receptor in immune organs, Melatonin is biological processes recurring naturally hormone synthesized in most the pineal gland to blood of mammals also is synthesis in deferent cells, tissues and organs like lymphocytes, skin, eyes and gastrointestinal duct (⁵). Melatonin has been examination studies in parasitic, virus and bacterial infestations (⁶). Act the melatonin to promote antigen display, phagocytic activities and production of monocytes (⁷). Melatonin have important immune-modulatory effects e.g. Plasmodium that hepatocytes colonies and red blood cells will causes in death of malaria through that melatonin have precursors derived from the tryptophan will calcium release and modulate the cell cycle of P. falciparum (⁸). The melatonin treatment with Schistosoma mansoni act on decrease oxidative injury and increase permanence of hamster infected (⁹).
The goal of this study to specify influence of melatonin drug against *Enterobius vermicularis* by examined in rats.

**Materials And Methods**

**Collection eggs of *Enterobius vermicularis***:

Eggs were collected from infected children of school in Al-Najaf city, gathered in anus they suffer from anal itching by transparent adhesive tape (10), these eggs incubation at 36ºC in wet flask for 5 days, most eggs were ivied released through vexation of the body, these eggs contained within larva notice circulation movement after three from incubated inside shell, some of them hatch naturally as expressed (11) kept unit used in the experiment.

**Preparation of Melatonin Solution**

Consider melatonin slightly soluble in water so used dimethyl sulfide and ethanol (DMSO/Germany) to dissolve. Take 2 mg / milliliter 99.9 DMSO-melatonin were prepared as stock solution (12).

**Preparation and Infection Animals**

90 male wistar rats were weight 100-110 g kept under light period 12h light and 12h dark where divided into three groups each group contain 30 rats were placed in plastic cages contain food and water with a floor furnished with sawdust, good ventilation and continuous cleaning of the cages, 500 eggs number within movement larva examined under microscopic were counted from eggs sedimentation by slide chamber, group one were 30 rats infected with 500 egg of *E.vermicularis* only orally as control without drug, group two (Former) were 30 rats inoculated melatonin pretreated for 7 days before the infection daily orally at dose of 15 mg / kg body weight where dissolved in distilled water then give oral 500 eggs for 30 day and group three (Accompanying) inoculated melatonin with eggs daily at dose of 15 mg / kg body weight give oral 500 eggs for 30 days. Three groups were examining the stool after 10, 20 and 30 days of infection by microscope.

**Histology Animals**

Rats were numbness with 2.5 pentobarbital and postmortem, intestine were reapers then inglorious 10 formaldehyde to make a histological section of the infection and treated, eosin-haematoxylin stain then examined by microscope in magnification of 100x (13).

**Statistical Analysis**

Results were calculated by analyses data the one way by ANOVA test and statistical significance between groups analyses when (P < 0.05).

**Results**

As shown in table (1), there is a significant reduce in *E.vermicularis* infection in rats treated with melatonin former at the dose of 15 mg/kg which were 1 and 0 for eggs and worms respectively, while there is a significant decrease in *E. vermicularis* infection in rats treated with melatonin accompanying at a dose of 15 mg/kg which were 21 and 10 for eggs and worms respectively, both after 30 day of treatment compared with control without treated were 390 and 495 for eggs and worms respectively after 30 day of infection. This may due to protective effect of melatonin is put off the appearance of disease, retard death and reduce the mortality rate.

**Table 1: Influence of Melatonin drug on count of Eggs & Worms of *Enterobius vermicularis* in rats per 20 microscope fields / days.**

<table>
<thead>
<tr>
<th>Dose Mg/kg</th>
<th>10days</th>
<th>20days</th>
<th>30days</th>
<th>F P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs</td>
<td>Worms</td>
<td>Eggs</td>
<td>Worms</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>500</td>
<td>473</td>
<td>470</td>
<td>480</td>
</tr>
<tr>
<td>Melatonin Former 15 mg/kg</td>
<td>213</td>
<td>92</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>Melatonin Accompanying 15 mg/kg</td>
<td>322</td>
<td>211</td>
<td>105</td>
<td>57</td>
</tr>
</tbody>
</table>

LSD : Least Significant Difference
Discussion

Enterobiosis is a human intestinal parasitic disease caused by pinworm infects a lot of people especially children causes symptoms e.g. anal itching, painful or difficult urination, irritation, insomnia repeated infection causes weakened immunity and may lead to death in the absence of treatment (14). Because of resistance to conventional drug and repeated infection, must search for alternative drugs and low toxicity (15). In the present study used melatonin drug is suggested that can therapeutic differ agent like immune enhance functions, antioxidant effect, bacterial, fungi viral, and parasites infections, shown significant reduce *E. vermicularis* with melatonin former when dose 15 mg/kg were 1 and 0 for eggs and worms respectively while significant decrease *E. vermicularis* with melatonin accompanying when dose 15 mg/kg were 21 and 10 for eggs and worms respectively, both after 30 day of treatment compared with control without treated were 390 and 495 for eggs and worms respectively after 30 day of infection, this indicates that give melatonin former enhances of the immune response, as in Table 1.

This may due to protective effect of melatonin is put off the appearance of disease, retard death and reduce the mortality rate (16), these study consistent with (17) that melatonin have control through of experimental the *Trypanosoma cruzi* infection and lead to reduce the parasitemia levels in rats. Another reported by (18) that melatonin drug cellular immunity activity by increased production lymphocyte in *Toxoplasma gondii* infected in rats. As in other study show reduce *Leishmania* infection to 40 in hamsters infected during the when serum melatonin being high compare to animals infected when melatonin level being low, this indicates that melatonin receptors plays an important role in leishmaniasis treatment (19).

As shown in the current study, it have been seen in histological analysis for untreated section granuloma fashioning in the intestine, necrosis, adenoma and hemorrhage of the bowel (20).

There was a statistically increased numbers of leucocytes production which observed in both the accompanying and former melatonin treatment observation tissues necrosis scatter among regions and inflammatory cells sneak shrill comparison with *E. vermicularis* infection only (P < 0.05) may due to melatonin increased immune-modulatory activates and have ability on stimulate innate immune cells in positive attachment between melatonin and phagocytic efficacy with infected (21).

This study agreed with (22) showed that exogenously manage melatonin significant reduced the amoebic necrosis areas also increased of leukophagocytosis and number of the dead amoebae.

In other study *Trypanosoma brucei* parasite was given the melatonin infected rats make histological changes in pineal gland where caused in reduce plasma level which may due to release of inflammatory mediators and become not inroad cell (23).

**Ethical Clearance**: Taken from University of Kufa ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Dynamic Changes in Salivary Cortisol and Protein among Dental Students

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Abstract

Background: Student assessments are the traditional methods of assessing academic success, and they are considered to affect one’s career. This study evaluated the levels of salivary stress biomarkers represented by cortisol and total salivary protein during final academic assessments of dental undergraduates.

Methods: Saliva samples were obtained, one before the exam another afterwards. Concentrations of salivary stress biomarkers were obtained by enzyme-linked immunosorbent assay (ELISA).

Results: Before the exam, the two parameters were dramatically higher than afterwards, with a substantial difference between the levels of salivary protein and cortisol (p=0.000, 0.000 consecutively).

Conclusion: Stress induced by academic examinations may increase the level of salivary stress biomarkers in the short term.

Keywords: Academic stress; cortisol; ELISA; saliva; salivary stress biomarkers

Introduction

Academic exams are considered to be among the most challenging experiences for students, since passing or failing typically has implications for career development. In particular, two primary systems or locations in the brain are involved in the stress response: the sympatho-adrenomedullary and system hypothalamus-pituitary-adrenocortical axis. Increased secretion of cortisol in the adrenal cortex occurs due to the activation of HPA. Therefore, salivary cortisol represents the activity of HPA and is a more effective assessment than blood collection in stress research, which can induce spurious increases in cortisol secretion, representing the hyper-stress component. A wide range of data has revealed that several kinds of psychological stress can result in HPA activation, leading to cortisol release and subsequently significantly higher salivary cortisol levels than resting baseline levels. The principal glucocorticoid in the human adrenal cortex is cortisol, which is synthesised from cholesterol. Higher levels of cortisol as a response to biochemical stress contribute to the well-characterised suppression of HPA related to health events and cognition. Salivary cortisol is present in a stable, unbound form and is the only fraction of hormones that display metabolic activity in combination with unbound plasma cortisol in the blood. Unbound cortisol reaches cells through passive diffusion due to lower of molecular weight and lipophilic nature, making it is possible to measure free cortisol in many body fluids. Up to 95% of secreted cortisol attaches to large protein molecules, like albumin and it is transported in the blood in the body. Salivary proteins have important functions, including the health of the oral cavity; the nutrition, survival and colonisation of microorganisms; and the adhesion and aggregation of microorganisms. Moreover, greater concentrations of total protein in response to stress lead to changes in saliva chemical properties, including oral surface adhesion or lubrication,

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and viscosity. Stress levels vary in different areas of education and learning, and higher stress levels could undermine the cognitive functions and learning abilities of students. Moreover, the prevalence of depression, anxiety and psychological distress among medical students is increasing. To investigate this phenomenon, this study used salivary cortisol as a biomarker for stress assessment during academic examinations, with higher levels found prior to a written test and its anticipation. Many studies have reported a rise in stress hormones (levels of cortisol) in anticipation of stressful experiences, such as oral exams, cardiac surgery and dental treatment. Moreover, the response to physical or psychological stress in the body increases the cortisol secretion. Stress can be beneficial, of course, as it can boost drive and energy to get through stressful situations, such as examinations and work deadlines. Students face numerous academic problems in today’s highly competitive world, however, including exam stress, lack of interest in a class and failure to understand a subject. Exam stress is the feeling of fear or anxiety over one’s performance in examinations, and academic stress can increase students’ anxiety levels. Interest has been rising in identifying and using biomarkers in saliva as a more evaluative way to measure stress. The investigation of stress biomarkers has achieved recognition because saliva sample collection is standardised, non-invasive and easy to manage. Studies have shown that saliva can be used in chair-side tests for many oral and systemic diseases. Saliva is useful because of its many analytes that are affected by a variety of conditions and physiological and pathological stressors. Therefore, this study was designed to confirm salivary cortisol and total protein levels during psychological stress among undergraduate students in a dental college.

**Materials and methods**

From 2016–2017, a cross-sectional study has been conducted at the College of Dentistry, University of Anbar, Iraq. A total of 12 undergraduate students (6 males and 6 females) aged 21–23 years were randomly selected and recruited from different academic years to study. The study’s objective and protocol were explained to all the recruited students, and their voluntary consent was obtained prior to participation. Two unstimulated saliva samples were collected from each student. In order to decrease the presence of food debris and consequent salivation stimulation, students were asked not to eat and drink water only about an hour prior to sample collection. The first sample was taken 30 minutes before a written examination at 8:30 am, and the second sample was taken at 12:00 pm after the examination was complete. Each student was instructed to rinse his or her mouth to remove debris. De-ionised water was used to rinse the mouth, and the participants were then asked to spit for 5 minutes in a special sampling container. The container was labelled with a collection number (1 or 2), date and time. All salivary samples were centrifuged at 3,000 rpm for 10 min to isolate pure saliva. Total protein and cortisol concentrations were calculated using a special kit (SPINREACT, Spain) with an enzyme-linked immunosorbent assay, as directed by the manufacturer.

**Statistical Analyses**

The data analysis was performed using version 11.0 of SPSS. A *p*-value smaller than 0.05 was determined as statistically significant. To check for discrepancies in the categorical variables, a Chi-square test was used.

**Results**

A total of 12 dental students (6 male, 6 female) from different academic years were recruited for this research to measure their levels of total free salivary cortisol (ng/ml) and total protein content in saliva. As shown in Figures 1 and 2, both parameters were significantly higher before the exam than afterwards, with a substantial difference between salivary protein and cortisol levels (*p*=0.000, 0.000 consecutively).
Discussion

Saliva plays an important role in the maintenance of oral hygiene. Various studies have shown that salivation changes often occur due to stress. In this study, we correlated the levels of certain parameters in saliva with the stress induced by an examination in medical students. Protein levels in saliva were significantly higher before the examination than afterwards, a finding consistent with that of Nauvoma et al., who also showed a significant increase in protein concentrations in saliva immediately after stress exposure\(^\text{10}\). Another study by Al-Nuaimy et al. showed similar findings by estimating protein concentrations before an oral academic exam and after one month of holiday break\(^\text{11}\). To understand the mechanism behind this increased protein concentration, the activation of salivary glands as a sympathetic intervention during exposure to stress to control protein secretion has also been explored\(^\text{10, 12}\). The autonomic nervous system primarily controls protein secretion mainly by three salivary glands: parotid, sublingual and submandibular. The release of protein from the submandibular glands and parotid is elicited by stimulating the sympathetic system, and protein release from the sublingual gland is usually elicited by stimulating the parasympathetic system\(^\text{13}\).

We also measured free salivary cortisol levels during a stress condition before and after the academic exam. During chronic stress, alteration in cortisol levels is prominent. Cortisol is a stress hormone that is synthesised in the cortex of the adrenal gland\(^\text{14}\). As discussed earlier, the measurement of cortisol in saliva is superior to that in serum because a salivary assay enables the measurement of unbound cortisol.

We observed significantly higher cortisol levels in saliva before the academic examination in both males and females than afterwards, showing a direct correlation between saliva cortisol levels and stress.
Other studies have reported similar findings, indicating that training during medical courses causes higher incidences of psychological stress in students and that academic examinations are major stressors for students, likely because their performance affects their future careers\(^2\,\!15\). This increased stress leads to increased levels of cortisol\(^10,11,16\). The increase in cortisol levels is attributed to HPA axis activity, which is more intense during stress reactions. Hypothalamus-level stressors activate the secretion of CRH and AVP, which stimulate the frontal lobe of the pituitary gland to release adenocorticotroic hormone (ACTH). ACTH stimulates the synthesis of cortisol and its secretion in the adrenal cells. Stress reactions are exhaustive and damaging to the body; indeed, cortisol limits and minimises the catabolic and immunosuppressive effects of stress reactions through a negative feedback mechanism\(^17,18\).

This study therefore demonstrates that the stress of academic examination affected important components of saliva, including protein and cortisol, which decreased after the exam was over. This suggests that these levels are a short-term response to stress.

**Conclusion**

This study has shown increased protein and cortisol concentrations in saliva before the commencement of an academic exam, which decreased after the exam was over, suggesting that stress precipitates short-term changes in saliva composition. Higher levels of cortisol during an examination can reduce stress during the examination.

**Conflict of Interest:** All the authors declare no conflicts of interest.

**Funding:** N/A

**Acknowledgement:** We thank our dental colleagues at the College of Dentistry, University of Anbar, who aided the investigation.

**Ethical Approval:** The study was conducted in compliance with the Helsinki Declaration and with the approval of the Regional Committee on Ethics, which is represented by the Medical Ethics Committee of the Ministry of Health in Iraq.

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Prevalence of Smoking among Iraqi Female Medical Providers in Baghdad

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Abstract

**Background:** Cigarette smoking is the largest preventable risk factor for morbidity and mortality in developed countries. Healthcare providers who smoke are less likely to advise patients to quit smoking. Being a female and smoker adding more burdens on society.

The objective of the study is to assess the prevalence of smoking among Iraqi female medical provider. A descriptive cross-sectional study with an analytic element. Data collection was done via electronic questionnaire forms distributed online to contact list for 450 easy to reach female health providers (doctors, dentists, pharmacists) who work in different hospitals, PHCs and health institutes in Iraq. Nearly 15.3% of the samples were smokers, 53% of sample was 20-29 years while nearly 62% of sample was married. About two third of sample had bachelor degree (66.2) while nearly half of the sample (52.9) were doctors. Regarding the place of work about (55.6) of sample were working in hospital and the years of service were equally distributed between <5 years and >5 years. Nearly half of the sample has husbands or friends who are smokers. The smoking environment was significantly affecting the smoking status while there is no association between age, marital status, education, field of work, place of work and years of service. The percentage of female smokers among medical service providers is high, and female doctors got the highest rate. With regard to age, the age group between 20-29 was the highest among female smokers. The results also showed a higher percentage among those who work in hospitals, and the largest proportion of female smokers was among those who had spouses or friends who smoked.

**Keywords:** smoking, Iraqi female, medical provider

Introduction

Worldwide, tobacco use represents one of the major causes of death and the main preventable cause of lifestyle-related diseases, such as lung cancer, chronic obstructive pulmonary disease, and coronary heart disease (1). Smoking is a major preventable cause of morbidity and mortality (2). Smoking for anyone, at any age, is dangerous and can lead to preventable disease, and even death. But, for women, smoking carries certain additional risks (3). About 250 million women in the world are daily smokers. About 22 percent of women in developed countries and 9 percent of women in developing countries smoke tobacco. In addition, many women in south Asia chew tobacco (4). The tobacco industry promotes cigarettes to women using seductive but false images of vitality, slimness, modernity, emancipation, sophistication, and sexual allure. In reality, it causes disease and death. Tobacco companies have now produced a range of brands aimed at women. Most notable are the “women only” brands: these “feminized” cigarettes are long, extra-slim, low-tar, light-colored or menthol (4). Health care professionals play a prominent role in promoting tobacco control and smoking cessation programs. However, their smoking habits may prevent them from providing unbiased advice on smoking cessation and may even prevent them from being efficiently involved in cessation programs designed for patients (5). Physicians who smoke are less likely to advise patients to quit smoking. Also, it is less expected from them to assess patient’s will to refrain from smoking (6). This research throws a light on
smoking among female medical providers in Iraq, and aims to estimate its prevalence.

It is vital to assess health professionals’ smoking habits for two reasons. First, they have a direct effect on their health and wellbeing. Secondly, it has been shown that physicians who smoke tobacco are less likely to advise their patients regarding the health hazards of tobacco smoking (7).

**Methods**

A descriptive cross-sectional study with an analytic element. Data collection was done via electronic questionnaire forms distributed online to contact list for 450 -easy to reach- female health providers (doctors, dentists, pharmacists) who work in different hospitals, PHCs and health institutes in Iraq. The questionnaire included sociodemographic & occupational information’s of participants: age, marital status, no. of children, education, field of work, place of work, and years of service. Collected data were fed, statistically analyzed, presented using SPSS V.20.

**Results**

The distribution of the sample by sociodemographic variables is presented in table 1: About 53% of sample was 20-29 years old while nearly 62% of sample was married with nearly (69.1%) of the sample had number of children between (0-2). About two thirds of sample had bachelor degree (66.2%), while nearly half of the sample (52.9%) was doctors. Regarding the place of work (55.6) of sample were working in hospital and years of service were equally distributed between <5 years and >5 years. According to table 2: (15.3%) of the sample was smokers. Nearly half the sample has smoking husbands or friends. The smoking environment was significantly affecting the smoking status, while according to table 3 there is no statistical association between smoking with age, marital status, number of children, education, field of work, place of work or years of service.

Nearly half (53.6%) smokers reported enjoyment as the main reason for smoking, while non-smoking reasons for the majority (64.6%) were fear from health risks (table 4).

Vast majority of smokers (72.4%) were smoking any time, 55.07% of smokers were smoking at home only. Nearly half of the smokers have tried to quit. Regarding symptoms suffered, 31.9% have teeth discoloration, 18.8% have changes in mouth odor, and 16% have shortness of breath. Both dry mouth and night cough were encountered in 14.5%, while only 13% have voice changes.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age class</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>237</td>
<td>52.7</td>
</tr>
<tr>
<td>30-39</td>
<td>170</td>
<td>37.8</td>
</tr>
<tr>
<td>+40</td>
<td>43</td>
<td>9.6</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
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<td></td>
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<td>277</td>
<td>61.6</td>
</tr>
<tr>
<td>Unmarried</td>
<td>173</td>
<td>38.4</td>
</tr>
<tr>
<td><strong>No of children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>311</td>
<td>69.1</td>
</tr>
<tr>
<td>3-5</td>
<td>99</td>
<td>22</td>
</tr>
<tr>
<td>+3</td>
<td>40</td>
<td>8.9</td>
</tr>
<tr>
<td>Education</td>
<td>Bachelor</td>
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</tr>
<tr>
<td>--------------------</td>
<td>----------</td>
<td>-----</td>
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<tr>
<td>Post graduate</td>
<td>152</td>
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</table>

<table>
<thead>
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<th>Pharmacist</th>
<th>95</th>
<th>21.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctors</td>
<td>238</td>
<td></td>
<td>52.9</td>
</tr>
<tr>
<td>Dentist</td>
<td>117</td>
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<td>26.0</td>
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</table>

<table>
<thead>
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<th>Health institute</th>
<th>111</th>
<th>24.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHC</td>
<td>89</td>
<td></td>
<td>19.8</td>
</tr>
<tr>
<td>Hospital</td>
<td>250</td>
<td></td>
<td>55.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Years of service</th>
<th>&gt;5</th>
<th>225</th>
<th>50.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>225</td>
<td></td>
<td>50.0</td>
</tr>
</tbody>
</table>

| Total             | 450|     | 100  |

Table 2: Social smoking environment

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>No family history</th>
<th>Parents and siblings</th>
<th>Husband and friends</th>
<th>Tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>5(7.3)</td>
<td>31(44.9)</td>
<td>33(47.8)</td>
<td>69 (15.3%)</td>
</tr>
<tr>
<td>Non smoker</td>
<td>106(27.8)</td>
<td>157(41.5)</td>
<td>118(31)</td>
<td>381 (84.7)</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>188</td>
<td>151</td>
<td>450 (100)</td>
</tr>
</tbody>
</table>

X² 15.166 df 2 P=0.001

Table 3: Smoking status according to socio-demographic & occupational variables of sample studied

<table>
<thead>
<tr>
<th>Age</th>
<th>Smoker</th>
<th>Non smoker</th>
<th>Total</th>
<th>X2</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>41(59.4)</td>
<td>196(51.4)</td>
<td>237</td>
<td>1.899</td>
<td>2</td>
<td>0.387</td>
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<tr>
<td>30-39</td>
<td>21(30.4)</td>
<td>149(39.1)</td>
<td>170</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+40</td>
<td>7(10.2)</td>
<td>36(9.5)</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Smoker</th>
<th>Non smoker</th>
<th>Total</th>
<th>X2</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever married</td>
<td>44</td>
<td>233</td>
<td>277</td>
<td>0.169</td>
<td>1</td>
<td>0.681</td>
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<tr>
<td>Non married</td>
<td>25</td>
<td>148</td>
<td>173</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>No of children</th>
<th>Smoker</th>
<th>Non smoker</th>
<th>Total</th>
<th>X2</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>49</td>
<td>262</td>
<td>311</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>13</td>
<td>86</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>+5</td>
<td>7</td>
<td>33</td>
<td>40</td>
<td>0.557</td>
<td>2</td>
<td>0.757</td>
</tr>
</tbody>
</table>
Cont.. Table 3: Smoking status according to socio-demographic & occupational variables of sample studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>Post graduate</td>
<td>18 (53.6)</td>
</tr>
<tr>
<td>bachelor</td>
<td>152 (34.6)</td>
</tr>
<tr>
<td>Field of work</td>
<td></td>
</tr>
<tr>
<td>Doctors</td>
<td>35 (50.7)</td>
</tr>
<tr>
<td>dentists</td>
<td>22 (31.9)</td>
</tr>
<tr>
<td>pharmacists</td>
<td>12 (17.4)</td>
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<tr>
<td>Place of work</td>
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<tr>
<td>PHC</td>
<td>8 (53.6)</td>
</tr>
<tr>
<td>Hospital</td>
<td>42 (53.6)</td>
</tr>
<tr>
<td>Health institute</td>
<td>19 (53.6)</td>
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<tr>
<td>Years of service</td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>36 (53.6)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>33 (53.6)</td>
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Table 4: Reasons of smoking and non-smoking

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Reasons</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>Enjoy</td>
<td>37 (53.6)</td>
</tr>
<tr>
<td></td>
<td>Stress</td>
<td>21 (30.4)</td>
</tr>
<tr>
<td></td>
<td>With smoker group</td>
<td>7 (10.1)</td>
</tr>
<tr>
<td></td>
<td>Habit</td>
<td>3 (4.3)</td>
</tr>
<tr>
<td></td>
<td>Adult feeling</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>69 (15.3)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>Health risk</td>
<td>246 (64.6)</td>
</tr>
<tr>
<td></td>
<td>Belief</td>
<td>83 (21.8)</td>
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<tr>
<td></td>
<td>Cultural barrier</td>
<td>46 (12.07)</td>
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<tr>
<td></td>
<td>Husband refusal</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td></td>
<td>Subtotal</td>
<td>381 (84.7)</td>
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<tr>
<td>Total</td>
<td></td>
<td>450 (100)</td>
</tr>
</tbody>
</table>

Table 5: Smoking setting & consequences

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
</tr>
<tr>
<td>Cigarette</td>
<td>45 (65.3)</td>
</tr>
<tr>
<td>Nargileh</td>
<td>24 (34.7)</td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td>Anytime</td>
<td>50 (72.4)</td>
</tr>
<tr>
<td>Morning</td>
<td>0 (0)</td>
</tr>
<tr>
<td>At night</td>
<td>19 (27.6)</td>
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</table>
Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1

**Cont.. Table 5: Smoking setting & consequences**

<table>
<thead>
<tr>
<th>Place</th>
<th>At home</th>
<th>Workplace</th>
<th>Anywhere</th>
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<tr>
<td></td>
<td>38</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>55.07</td>
<td>2.8</td>
<td>42.03</td>
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</table>

<table>
<thead>
<tr>
<th>Try to quit</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>55.07</td>
<td>44.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Dry mouth</th>
<th>Teeth discoloration</th>
<th>Mouth odor</th>
<th>Voice change</th>
<th>Night cough</th>
<th>Shortness of breath</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>22</td>
<td>13</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>14.5</td>
<td>31.9</td>
<td>18.8</td>
<td>13</td>
<td>14.5</td>
<td>15.9</td>
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</tbody>
</table>

**Discussion**

Tobacco smoking is responsible for >7 million deaths per year, nearly 80% of which occur in low- and middle-income countries\(^8\). The percentage of smokers among studied sample was relatively high compared to (1.9\%) and (13.2\%) in Hilla city/Iraq\(^9\) and India\(^10\) respectively.

Doctors show the highest percentage of smokers (50.7\%). While the higher percentage of smoking (52.7\%) was in (20-29) years old. This may be due to the fact the young female medical providers have liberal thoughts about smoking and do not consider it as stigma, besides the openness to neighboring countries, in addition to spending more time on internet. In this study the minority of the sample were postgraduate, this due to the fact that the majority of the sample were young in age. About (55.6\%) of the sample are working in hospitals this also can be due to the same reason which is the smallest age of the doctors, dentists and pharmacists the higher possibility of working in hospitals as interns or permanent resident. That is also applicable on years of service.

Coming to the smoking environment, it was the only significant variable associated with smoking status. This agrees with a study in USA in which they found that having two ever-smoking parents, in comparison to zero or one, was associated with higher nicotine dependence scores, cigarettes per day\(^14\) which may be explained by the strong influence of family and friends on customs and temperament.

The present study reveals that the higher percentage of smoking reason (53.6\%) was enjoyment. This agrees with a study in Saudi Arabia\(^12\). This may be explained by the fact that doctors’ life is stressful.

Regarding the smoking characteristics about (65.3\%) of smokers smoked cigarettes. About half of them smoked at any time while 55% smoked at home. Smoking at home is more convenient for female doctor’s giving privacy, and is more suitable than morning medical work, or afternoon clinic work. Nearly 55% of smokers have tried to quit. This is higher than a study in Armenia.

Coming to the side effects of smoking reveled in this study, the highest was (32\%) teeth discoloration, the rest of side effects were change of mouth odor, shortness of breath, dry mouth, night cough and voice changes.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required
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Retrospective Study of Acute Pediatric Intoxication Cases by Household Products Presented to the Poison Control Center of Ain-Shams University Hospitals

Sherien Salah Ghaleb¹, Lamiaa Ewis Abd Alfatah², Hoda Sayed Mahmoud³

¹Professor of Forensic Medicine and Clinical Toxicology, Cairo University, ²Assistant Lecturer of Forensic Medicine and Clinical Toxicology, Beni-Suef University, ³Lecturer of Forensic Medicine & Clinical Toxicology, Beni-Suef University

Abstract

Background: Acute poisoning in children is a crucial pediatric emergency and may be a worldwide problem. This study aims to acknowledge the incidence of acute poisoning by household products in children regarding demographic factors, common clinical presentation and outcome of management.

Methods: this is often a descriptive retrospective study conducted on patients admitted to the Poison Control Centre of Ain-Shams University Hospital. The duration of the study was one year, from the beginning of January, 2016 till the top of December, 2016. the entire number of cases was 846 cases collected and analyzed regarding the demographic data, condition of poisoning, common clinical presentation, and management plan. Data was analyzed using computer software package SPSS 15.

Results: a complete of 846 cases were reviewed, the varied age groups involved ranged from but one year to 18 years, with a mean age of 10.22 ± 6.83 years. Most cases were females (67 %), living in urban areas (52.4 %) and therefore the majority of cases were accidental (74 %). the foremost common offending agent was pesticides (71%). Most of the patients were vitally stable on admission and therefore the commonest clinical presentation was gastrointestinal symptoms (31.3%). Most of cases received medical treatment within the inpatient wards (80.5%) and (96.7%) improved while (3.3 %) died.

Conclusions: Acute poisoning by household products is common among adolescents and pre-school age children. Pesticides were liable for the bulk of cases. Supportive and symptomatic therapy is that the main method for treatment.

Keywords: acute poisoning, children, household products, pesticides.

Introduction

Acute poisoning is a common situation in the emergency departments (EDs) all over the world and involves high medical attention and significant costs.¹ Childhood poisoning is a significant public health problem and a preventable cause of morbidity and mortality.²

The most important difference between pediatric and adult poisoning is types of agents. In adults, higher percentages of poisoning cases are due to psychopharmacologic drugs (sedatives, tranquilizers and antidepressants), whereas in children, there is a much higher frequency of exposure to household items and personal care products and plants.³

Many studies indicated that a variety of social and demographic factors like family size, socioeconomic condition, attention to child as well as storage place of poison are important risk factors which significantly influence the acute household poisoning cases in children.⁴

Accidental poisoning has a strong age predilection. This problem is particularly common in toddler and
Materials and Methods

This is a descriptive retrospective study. Data of all acute toxicity cases by household products among children (total number of cases was 846), who were admitted to the Poison Control Centre of Ain-Shams University Hospital (PCC- ASUH) during one year study period, from January, 2016 to December, 2016 were collected and analyzed. Cases were categorized according to age, sex, residence, time of poison exposure, manner of toxicity, type of poison, presentations, management plan and final outcome of the cases. Patients were divided into 4 age groups; these are infancy (< 2 years), preschool age (2-6 years), school age (7-12 years) and adolescents (13-18 years). Residence of the patients was also classified into urban and rural areas. According to type of poison, the patients were also divided into 4 groups; these are pesticides group, cleaning and disinfectant products group, hydrocarbons group and miscellaneous group. General management steps (ABCs), Specific measures like decontamination, gastric lavage, administration of activated charcoal and antidotes were also recorded. Data was coded and entered using the statistical package for Social Sciences (SPSS version 15). The data were summarized using a descriptive frequency and percentage for quantitative values. Relation between data grouped was tested by Chi-Square test for quantitative variables. Statistical differences (P-values) less than or equal to 0.05 were considered statistically significant. Data was collected after obtaining consent from the chef of the PCC of ASUH and from the ethical committee of scientific research, Faculty of Medicine, Beni-Suef University.

Results

This study was conducted on 846 children. The various age groups involved ranged from less than one year to 18 years, with a mean age of 10.22 ± 6.83 years. The adolescent age group had the greatest representation (52.7 %), followed by pre-school age children (37.5 %), infants (6 %), and school age children (3.8 %) figure (1). Females were more common than males (67 %) figure (2). Most of children were living in urban areas (52.4 %).

As regard the type of agents involved, the pesticides group was the most common (71 %) and was distributed as follows: rodenticides (66.4 %), insecticides (33.6 %). Followed by cleaning and disinfectant products group (18.9 %) which was distributed as follows: bleaches (70 %), sulfuric acid (15 %), carbolic acid (6.2 %) and flash (8.8 %). Then the group of hydrocarbons (5.2 %) showed that the cases of kerosene were 95.5 % and those of other hydrocarbons were 4.5 %. Lastly the miscellaneous group (4.9 %) and was distributed as follows: cosmetics and personal care products were 92.7 % and others were 7.3 % figure (3).

Regarding the manner of toxicity, the majority of cases were accidental (74 %) while suicidal poisoning was in (26 %). Accidental poisoning was more common than suicidal among all age categories and was more common in males (85.7 %) than females (68.3 %) while suicidal poisoning was more common in females (31.7 %) than males (14.3 %). Suicidal cases were reported only during Adolescence table (1). It was found that all types of household products toxicity was more common in females than males except for hydrocarbons group; males were more common than females (66 %) Vs (34 %) table (2).

Regarding the clinical manifestation, the most common clinical presentation was gastrointestinal symptoms (31.3 %) figure (4), neurological symptoms was in (22.3 %) of patients, respiratory symptoms (10.8 %), Cardiovascular symptoms (3.3 %), Multiple symptoms (5 %) and others (1.8 %).216 (25.5 %) patients were asymptomatic.

Most of the patients were vitally stable on admission and had normal serum sodium, potassium and blood glucose level at presentation time. However, hypokalemia was detected in (26.7 %) figure (5).

According to the place of admission, the study revealed that most patients received medical treatment in the inpatient wards (80.5 %) followed by the intensive care unit and those observed in emergency department without admission (15.7 % and 3.8 %) respectively table (3).
Treatment of cases mainly depends on supportive and symptomatic treatment, elimination of the poison from the body and the use of antidote if available. For airway and breathing, oxygen was used in (4.8 %), endo-tracheal tube was inserted in (5 %) and only (3.4 %) of patients were put on mechanical ventilation. For circulation, majority of patients received IV fluids (97.3 %), steroids (11.7 %) and dopamine (0.9 %). For symptomatic treatment, antibiotics were used in (0.2 %), anti-emetics (56.8 %), H2 blockers (29 %), bicarbonate (8.9 %), sedative hypnotics (1.9 %) and epanutin (0.1 %).

Regarding GIT decontamination and the use of physiological antidote, Activated charcoal was used in (5 %) of patients and Gastric lavage (13.1 %). Antidotes were given to treat 42.2 % of cases. Atropine was the most common antidote used in 26.5 % of cases, Toxoguonin (15.4 %) and NAC (0.3 %).

Regarding the outcome of the patients, (96.7 %) improved when received medical treatment and discharged while (3.3 %) died. The highest mortality was in hydrocarbons group (9.1 %) followed by pesticides group (3.3 %) then cleaning & disinfectant products group (2.5 %).

**Discussion**

The demographic data of the present study revealed a highly significant increase within the incidence of acute poisoning by household products among patients in Adolescence period aged 13-18 years (52.7%) followed by Pre-school group aged 2-6 years (37.5%) an equivalent as observed in other studies.6,2 the bulk of cases were females (67%), while males were (33%) this is often almost like other studies.7 Children belonging to urban areas were more exposed (52.4%) compared to those in rural areas (47.6%) this might flow from to the very fact that mothers in populated area are busier in their jobs and resulting in neglect of their child during this area. The toxic agents can also be more available within the cities than within the rural areas.8 this study indicated that the pesticides group was the foremost common explanation for poisoning (71%) followed by cleaning and disinfectant products group (18.9%) These results are approximately almost like other studies8. Regarding time of poisoning, most of poison cases were in evening (46 %) then afternoon (32 %) This agrees with other studies.10,11 Accidental poisoning was more common than suicidal among all age categories while suicidal poisoning was more common in females (31.7%) than males (14.3%). These results agreed with other studies.12 The bulk of patients were vitally stable. Gastrointestinal symptoms (vomiting, abdominal colic, diarrhea, dysphagia & hematemesis) were the foremost common symptoms (31.3%) followed by neurological symptoms (22.3%). These results are almost like previous study.5 The majority of patients had normal sodium, potassium and blood sugar level. However, hypokalemia was detected in (26.7 %).13 Most of patients received medical treatment within the inpatient wards (80.5%) followed by the medical care unit and people observed in emergency department without admission (15.7% and 3.8%) respectively.14 activated carbon was utilized in (5 %) of patients and lavage (13.1 %). Antidotes got to treat 42.2 % of cases. Atropine was the foremost common antidote utilized in 26.5 % cases, Toxoguonin (15.4 %) and NAC (0.3 %) (15). while 28 (3.3 %) of our patients died, (96.7 %) improved when received medical treatment an equivalent as observed in other studies.16

**Conclusion**

Acute poisoning is a crucial explanation for emergency unit admissions. The incidence of poison exposure was highest among adolescents and pre-school age children. Intentional poisoning was more common in older girls and accidental poisoning was more common in younger boys. Pesticides and household cleansing products were liable for the bulk of cases of poison exposure. Gastrointestinal symptoms were the foremost common clinical presentations in acute toxicity by household products. In most of cases, treatment was non-specific, including general decontamination and supportive-symptomatic therapy. The utilization of physiological antidote is restricted to pesticides toxicity.

**Funding :** Not applicable as no fund was obtained for the study.

**Availability of data and materials** Please contact author for data requests

**Authors’ contributions**

All authors read and approved the final manuscript.

**Ethics approval and consent to participate**
Ethical approval was obtained from the chef of the poison control Centre of Ain-Shams University hospitals to collect the data from the archives of the patients’ files in the Centre.

Consent for publication: Not applicable as no individual data, images or videos were included in the study.

Conflict of Interest: Nil

References


Maternal Comorbidities Associated with Preterm Deliveries in Comparison with Full Term Delivery in Al-Zahraa Teaching Hospital in Al Najaf City

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Abstract

Background: Preterm birth is one of the major conditions that affect on infant mortality and morbidity, many of maternal comorbidities effect on the fetus outcome, in this study we evaluate some of maternal condition that effect on preterm birth and compare them with full term birth to know the most common factors associated with this condition to decrease the rate of preterm birth and reduce neonatal mortality and morbidity.

Methods: A case control study was conducted at first of April to thirty of September 2018, in Al Zahra Teaching Hospital in Al Najaf City, 300 delivered pregnant women were participated divided to 100 cases as preterm delivery women and 200 controls as full term deliver women enrolled in the study, maternal comorbidities were recorded and binary regression analysis was used for analysis of the study.

Results: The study show many significant association between preterm birth and maternal risk factors include, urinary tract infection (OR = 7.32), lower number of antenatal visit (OR=2.52), interval between pregnancy ≤2 year (OR =1.973), premature rupture of membrane (or =6.55), oligohydramnios (OR =6.55), gestational diabetes (OR =3.45), abruptio placentae (OR = 5.06) and previous preterm labor (OR=3.68).

Conclusion: Based on the results in the study the most determinants that affect on preterm birth were urinary tract infection, premature rupture of membrane and abruption placenta.

Keywords: Antepartum hemorrhage, Gestational Diabetes mellitus, Gestational Hypertension, Premature rupture of membrane, Preterm birth, maternal comorbidities, fetus outcome, oligohydramnios, abruptio placentae

Introduction

Preterm delivery defined by The World Health Organization (WHO) as infant delivered at time below 37 completed weeks of gestation. It was divided into the following categories depending on mother gestational age, extremely preterm (<28 weeks), very preterm (28–<32 weeks), moderate or late preterm (32–<37 completed weeks of gestation) [¹].

An about fifteen million babies are deliver too early every year. Nearly one million children die every year because of the complications of preterm birth [²].

Preterm birth (PTB) considered as a major cause of morbidity and mortality and its percentage are increasing with time in many countries [³, ⁴].
The majority of global preterm births occur in Asia and Africa with (85%), where the health systems are inadequate and weak [5, 6].

The rate of mortality, morbidity and the costs of Preterm labor are higher at lower gestational ages, in babies that survive, the risk is rise in form of short – and long term morbidity [7, 8].

Obesity may not directly lead to preterm birth [9] however; it may associate with diabetes and hypertension which are risk factors by them [8]. Marital status is also associated with risk for preterm birth [10]. Pregnancy outside of marital status was associated with a 20% rise in adverse outcomes [11].

Subfertility is another factor associated with preterm birth, Pregnancies after used in vitro fertilization confers a high risk of preterm birth after more than 1 year of trying [12].

A number of systemic maternal bacterial infections are associated with preterm birth this including pneumonia, appendicitis, pyelonephritis [13].

In study done in Iraq showed that risk of preterm birth increase with history of multiple pregnancy with (OR=7.5), history of cervical incompetence with (OR=4.7), and history of abortion with (OR=6.3) in comparison with pregnant women did not had previous history to such condition, heavy manual work with (OR = 1.70), and direct trauma to the abdomen (OR = 3.76) were also significantly associated with preterm birth [14].

A study done in Iran to measure risk factors of preterm birth found that history of preterm rupture of membrane, preeclampsia, and multiple pregnancies had increased risk of preterm birth with odd ratio equal 5.1, 4.6, and 17.4 respectively. In the same study the investigator found history of infertility and previous history of abortion were not statistically significant with preterm birth [15].

Another studies done in Ethiopia revealed that many factors associated with preterm birth include preterm rupture of membrane, maternal age more than 35 years old, poor antenatal care, and infection of pregnant women with HIV [16, 17].

A case–control study done in western part of China appeared that number of antenatal care four or less are more significantly associated with preterm birth with (OR=4) in relation to pregnant with adequate antenatal visit, while income of family, age of pregnant mother, and level of education were not significantly associated with preterm birth [18].

In Northwestern Russia study was investigate associations between preterm birth and selected maternal factors and found young pregnant with age (<18 years) or older (≥35 years), underweight, obese mothers, smoking status, abuse of alcohol and history of diabetes mellitus or gestational diabetes were more likely associated with preterm birth [19].

Approximately 45–50% of preterm deliveries are without cause, 30% are caused by (PROM) and other 15–20% is due to indicated or elective preterm deliveries. [20]

Although many years of research to determine the etiology, epidemiology, and management of preterm birth. The incidence of it has continued to increase. There are a lot of hypothesis found to explain the increase in preterm as a technology of assisted reproduction and the desire of obstetricians to use elective delivery of infant of pregnant in whom medical, fetal, or obstetrical complications happen preterm [21, 22, 23, 24].

Subjects and Methods:

Study design:
A case control study.

Study setting and time:
Study was carried out in Al Najaf city and data were collected from post delivery ward in Al-Zahraa Teaching Hospital which is a major teaching and referral hospital for obstetrics and gynecology receive pregnant from central and peripheral area, handles with uncomplicated and complicated deliveries, data collected in period between 1st of April 2018 to 30th of September 2018.

Sample size:
To achieve the aim of the study, a case control study use with sample size calculated according to equation below:
Sample size=
\[
\frac{r+1}{r} \frac{(P^r)(1-P^r)(Z_\beta^2 + Z_\alpha^2/2)}{(P_1-P_2)^2}
\]

Data collection:
The inclusion criteria include:
- Female at reproductive age 15-45 years.
- Singleton pregnancy determined by ultrasound.
- Pregnant know her LMP or had U/S at 1st trimester.

Exclusion from this study:
- Multiple gestations.
- Those who are using assisted conception.

Statistical Analysis
Data were entered, and analyzed using the statistical package for social sciences SPSS version 25.

Results
The parity of the women in both study groups showed no significant association between both groups, 34% vs. 26.5%, respectively in nulliparous women, and 66% vs.73.5% respectively in multiparous women (P = 0.224) with CI 95% of OR=(0.84 - 2.40).

The interval between pregnancies was 2 years or less in 44 women (66.7%) out of the 66 parous women (parity one or more) in preterm group and it was more than 2 years in the remaining 22 (33.3%), while the corresponding frequencies in the term group were 74 (50.3%) and 73 (49.7%) out of 147 parous women in this group respectively, statistically significant difference had been found (P= 0.027) (OR=1.97). History of preterm labor was significantly frequent in preterm than term group, 24.2% and 12.9%, respectively, (P=0.039) with (OR=2.15).

Previous cesarean sections (CS) was not significantly different between studied groups, 37.9% in preterm and 36.1% in term group, (P>0.05), CI 95% of OR= (0.59 - 1.97).

Number of antenatal visit was significantly lower in preterm than term group (P=0.037) where 21% of women in preterm had no antenatal visit compared to only 14% in the term group, additionally, 35 women (35%) in the preterm group had 1-3 visit compared to 58 (26.5%) in the term group while 44% preterm women and 59.5% term women had 4 or more visits. All findings regarding the obstetrical history of the studied groups are demonstrated in (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preterm (N=100)</th>
<th>Term (N=200)</th>
<th>P. value</th>
<th>OR</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>34</td>
<td>34.0</td>
<td>53</td>
<td>26.5</td>
<td>0.224</td>
</tr>
<tr>
<td>Multiparous</td>
<td>66</td>
<td>66.0</td>
<td>147</td>
<td>73.5</td>
<td></td>
</tr>
<tr>
<td>Interval between pregnancies*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 year</td>
<td>44</td>
<td>66.7</td>
<td>74</td>
<td>50.3</td>
<td>0.027</td>
</tr>
<tr>
<td>&gt;2 year</td>
<td>22</td>
<td>33.3</td>
<td>73</td>
<td>49.7</td>
<td></td>
</tr>
<tr>
<td>Previous preterm labour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>24.2</td>
<td>19</td>
<td>12.9</td>
<td>0.039</td>
</tr>
<tr>
<td>No</td>
<td>50</td>
<td>75.8</td>
<td>128</td>
<td>87.1</td>
<td></td>
</tr>
<tr>
<td>Previous CS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>37.9</td>
<td>53</td>
<td>36.1</td>
<td>0.798</td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>62.1</td>
<td>94</td>
<td>63.9</td>
<td></td>
</tr>
<tr>
<td>Number of antenatal visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>21</td>
<td>21.0</td>
<td>28</td>
<td>14.0</td>
<td>0.037</td>
</tr>
<tr>
<td>1-3</td>
<td>35</td>
<td>35.0</td>
<td>53</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>4 and more</td>
<td>44</td>
<td>44.0</td>
<td>119</td>
<td>59.5</td>
<td></td>
</tr>
</tbody>
</table>

*Primi women were excluded from calculation
In (Table 2) no significant differences had been observed between both groups regarding the mode of delivery or the sex of the neonate, (P>0.05) with CI 95% of OR= (0.75 - 2.02) and (0.41 - 1.08) respectively. Furthermore, all neonates in preterm group had birth weight of < 2500 gm. compared to only 6 (3%) in term group with a statistically significant difference (P<0.001).

### Table 2. Mode of delivery and neonatal characteristics of the studied group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preterm (N=100)</th>
<th>Term (N=200)</th>
<th>P. value</th>
<th>OR</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>41</td>
<td>41.0</td>
<td>72</td>
<td>36.0</td>
<td>0.399</td>
</tr>
<tr>
<td>VD</td>
<td>59</td>
<td>59.0</td>
<td>128</td>
<td>64.0</td>
<td></td>
</tr>
<tr>
<td>Sex of neonate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>48.0</td>
<td>116</td>
<td>58.0</td>
<td>0.101</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>52.0</td>
<td>84</td>
<td>42.0</td>
<td></td>
</tr>
<tr>
<td>Weight of neonate (gram)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; 2500</td>
<td>100</td>
<td>100.0</td>
<td>6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>≥ 2500</td>
<td>0</td>
<td>0</td>
<td>194</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows the comorbidities reported in both studied groups; where no statistically significant differences had been found in the frequency of chronic hypertension; 3% vs. 1.5%, (P>0.05), CI 95% of OR=(0.46 - 11.76) , gestational hypertension found in 14% and 11% of preterm and term groups, respectively, (P>0.05), CI 95% of OR=(0.72 - 3.04). Preeclampsia/eclampsia status was significantly more frequent in preterm group, (8%) compared to only (0.5%) in term group (P = 0.001) with (OR=18.56). Presence of cardiovascular disease was not significantly different between the studied groups, (P>0.05). Preexisting diabetes mellitus was not significantly different between both groups, (P>0.05), CI 95% of OR= (0.11 - 13.14), while gestational diabetes mellitus was significantly more frequent in preterm group (19%) than term group (10%), (P=0.001) with (OR=4.46). Frequencies of thyroid diseases and anemia were not significantly different between both groups (P >0.05) , CI 95% of OR= (0.22 - 8.33) and (0.67 to 1.76) for hypothyroidism and anemia respectively. Frequency of antepartum hemorrhage due to abruptio placentae was significantly higher in preterm than term group; 27% vs. 6%, (P=< 0.001) (OR=6.07) , APH due to placenta previa was not significantly different between groups, 7% in preterm and 5% in term group (P>0.05), CI 95% of OR=(0.69 - 5.16). Preterm rupture of membrane was significantly more frequently occurred in preterm group, (25%) than in term group (15%) (P<0.001) (OR=21.89). Polyhydramnios amniotic fluid status was not significantly different between both groups (P>0.05), CI 95% of OR= (0.56 - 6.39) while oligohydramnios was significantly more frequent in preterm than term group, 15% vs. 6%, respectively (P = 0.016) (OR=2.48).

Cervical incompetence was significantly more frequent in preterm group, (11%) compared to only 3% in term group, (P=0.005) (OR=4). Uterine abnormalities were not significantly frequent in preterm compared to term group, 2% vs. none, respectively (P=0.209). No significant difference between both groups in the presence of genital infection (P>0.05), CI 95% of OR= (0.57 - 1.49). Finally, higher proportion, 62%, of women in preterm group had (UTI) compared to 21.5% among women in term group, the difference was statistically significant, (P<0.001) (OR=5.96).
Table 3. Comorbidities reported among the studied group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Term (N=200)</th>
<th>P. value</th>
<th>OR</th>
<th>CI 95% of OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>Gestational</td>
<td>14</td>
<td>14</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Preeclampsia/eclampsia</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>None*</td>
<td>75</td>
<td>75</td>
<td>174</td>
<td>87</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>99</td>
<td>99</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preexisting</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Gestational</td>
<td>19</td>
<td>19</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>None*</td>
<td>80</td>
<td>80</td>
<td>188</td>
<td>94</td>
</tr>
<tr>
<td>Thyroid disease</td>
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<tr>
<td>Hyperthyroidism</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Hypothyroidism</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>None*</td>
<td>96</td>
<td>96</td>
<td>197</td>
<td>98.5</td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;11gm/dl</td>
<td>56</td>
<td>56</td>
<td>108</td>
<td>54</td>
</tr>
<tr>
<td>≥11gm/dl</td>
<td>44</td>
<td>44</td>
<td>92</td>
<td>46</td>
</tr>
<tr>
<td>APH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abruptio placenta</td>
<td>27</td>
<td>27</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>None*</td>
<td>66</td>
<td>66</td>
<td>178</td>
<td>89</td>
</tr>
<tr>
<td>Preterm rupture of membrane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>25</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>No</td>
<td>75</td>
<td>75</td>
<td>197</td>
<td>98.5</td>
</tr>
<tr>
<td>Amniotic fluid</td>
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</tr>
<tr>
<td>Polyhydramnios</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Normal**</td>
<td>80</td>
<td>80</td>
<td>182</td>
<td>91</td>
</tr>
<tr>
<td>Cervical incompetence</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>11</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>89</td>
<td>89</td>
<td>194</td>
<td>97</td>
</tr>
<tr>
<td>Uterine abnormality</td>
<td></td>
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<tr>
<td>Yes</td>
<td>2</td>
<td>2</td>
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<td>0</td>
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<tr>
<td>No</td>
<td>98</td>
<td>98</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Genital infection</td>
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</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>44</td>
<td>92</td>
<td>46</td>
</tr>
<tr>
<td>No</td>
<td>56</td>
<td>56</td>
<td>108</td>
<td>54</td>
</tr>
<tr>
<td>UTI</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>62</td>
<td>62</td>
<td>43</td>
<td>21.5</td>
</tr>
<tr>
<td>No</td>
<td>38</td>
<td>38</td>
<td>157</td>
<td>78.5</td>
</tr>
</tbody>
</table>

*None used as a reference subgroup in comparison **normal used as reference subgroup

Further analysis had been performed to assess the predictor factors of preterm labors, therefore, the factors that appeared to be significantly different between both groups were entered as independent factors (covariates) in
the bivariate regression analysis, and the status of birth (term or preterm) used as dependent variable and the test was run. Results of binary regression analysis and odds ratio (OR) are shown in (Table 4), where eight factors still significant and were the predictors associated with preterm labor after adjustment of other variables; these are interval between pregnancies less than 2year (OR = 1.973), previous preterm labor (OR= 3.68), lower no. of antenatal visit (OR =2.52), gestational diabetes mellitus (OR = 3.45), Abruptio placenta (OR = 5.06), preterm rupture of membrane (OR =6.55), oligohydramnios (OR = 3.53) and urinary infection (OR = 7.32), in all these factors, (P<0.05). Other factors showed no significant association (P>0.05).

Table 4. Results of binary regression analysis for the maternal factors associated with preterm labor

<table>
<thead>
<tr>
<th>Variable in the regression equation</th>
<th>B</th>
<th>Odds ratio (OR)</th>
<th>95% C.I. for (OR)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Interval between pregnancies (shorter)</td>
<td>0.680</td>
<td>1.973</td>
<td>1.077</td>
<td>3.614</td>
</tr>
<tr>
<td>Previous preterm labour</td>
<td>1.302</td>
<td>3.68</td>
<td>1.21</td>
<td>11.20</td>
</tr>
<tr>
<td>Lower no. of antenatal visit</td>
<td>0.926</td>
<td>2.52</td>
<td>1.46</td>
<td>4.37</td>
</tr>
<tr>
<td>Hypertension/preeclampsia-eclampsia</td>
<td>0.344</td>
<td>1.41</td>
<td>0.72</td>
<td>2.23</td>
</tr>
<tr>
<td>Gestational DM</td>
<td>1.239</td>
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<td>1.17</td>
<td>10.22</td>
</tr>
<tr>
<td>Abruptio placenta</td>
<td>1.622</td>
<td>5.06</td>
<td>2.48</td>
<td>10.34</td>
</tr>
<tr>
<td>Premature rupture membrane</td>
<td>1.879</td>
<td>6.55</td>
<td>1.34</td>
<td>32.06</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>1.262</td>
<td>3.53</td>
<td>1.43</td>
<td>8.74</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>0.259</td>
<td>1.30</td>
<td>0.21</td>
<td>8.04</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1.990</td>
<td>7.32</td>
<td>2.97</td>
<td>18.04</td>
</tr>
</tbody>
</table>

Discussion

In current study there is no significant association between PTB and parity, similar result present in Iraq [14], other study done in Iran had different result [25]. Also study done in Cairo found mothers with first baby had higher risk of preterm birth with (p=0.018) [26]. Other study done on preterm births which found that nulliparity is an important risk factor for preterm delivery in South Nigeria [27]. The discrepancy of finding in different countries may be attributed to the difference of the factors between the countries.

Interval between pregnancies of ≤ 2 year was found 2 fold more likely to cause PTB. This agree with study in Iran [25], these presentation may suggest that increase spacing between pregnancies could help to prevent the adverse pregnancy outcomes [28], while in Palestine no association present between PTB and interval between pregnancy [29].

The study also revealed a significant association of cervical incompetence and previous preterm delivery to cause PTB and this agree with study done in Palestine [29], and this could be the fact that the cervix is unable to maintain pregnancy to term.
In this study there is a significant association between number of antenatal visit (no visit) and the PTB in which 21% of preterm mother had no antenatal visit in comparison with 10% of full term mother, this agree with study done in Iran, with (p0.036) \[25\]. In a study done in Nigeria on the determination of preterm births, no booking of pregnant women in antenatal care program was found to be one of the strong determinants\[30\], while different result present in Kenya that found no association with preterm birth and attendance to antenatal care (p=0.621)\[31\].

The current study also investigate the possible association of preterm birth and history of HTN, in which preeclampsia/eclampsia was 19 fold more likely to cause PTB, while chronic and gestational hypertension not significantly associated with PTB, this agree with result in Iran found that the risk of preterm labor in mothers suffering from preeclampsia is 4.6 times higher than in other mothers\[32\]. Also other study in Tehran found preeclampsia and gestational HTN strongly associated with preterm birth\[33\]. Study done in japan demonstrated that the risk of preterm labor is higher in mothers suffering from preeclampsia or chronic hypertension \[34\]. The chronic and gestational HTN not appear as a risk factor this may attributed to well control of HTN in participant women.

In this study, from a statistical significance viewpoint, anemia was not associated with PTB p-value= (0.734), this agree with study in Palestine (p value=1.0) \[29\],also with study in Iran (P= 0.47) \[25\]. Disagreement appears in study done in Ethiopia \[35\] and in Cairo\[26\], the difference in significance of result may be attributed to degree of severity of anemia in studied women.

Previous cesarean section not significantly associated with pregnancy outcome (p=0.798), regarding to uterine abnormality, in this study was no statistically significant (P < 0.209) with PTB, and this result different from study done in Tehran\[33\]. Also no significant association between cardiovascular disease with (p=0.157) and thyroid disease (hyperthyroidism, hypothyroidism) with (p=0.208, p=0.889) respectively and pre term birth. No association in these factors may be due to need larger sample and more early investigations and fellow up.

The effect of gestational diabetes, it 4 fold increase risk related to preterm outcome p(0.001) while preexisting diabetes not significantly associated with preterm birth with (p=0.618). This agree with study done in china, found GDM is a significant risk factor with (OR=3.441) \[36\], and disagree with study done in Frances that found, pre-existing diabetes was strongly associated with PTB as a comparison with gestational diabetes \[37\]. In study done in al Mosul, no significant association present between diabetes mellitus and preterm birth \[14\]. In this study the preexisting diabetes not a risk factor, this my due to small number of participants detected .

Regarding antepartum hemorrhage the study found a significant association between placental abruption with preterm delivery, in contrast with placenta previa in relation with preterm delivery. Results also agree with study done in Iran that found no significant association between placenta previa and preterm birth \[25\]. Other study done in Palestine appeared that placenta abruption and placenta previa were found to be a significant risk factors for preterm birth\[29\]. In Nigeria study found a complication of pregnancy including antepartum hemorrhage was significantly associated with preterm birth \[38\]. Other research in Mosul reported that antepartum hemorrhage was not considered as a risk factor for preterm birth \[14\]. Placenta previa not significantly affect preterm this may attributed to the type of placenta previa like in study done by Dola et al. Found that preterm birth was more happened in pregnant women with complete placenta previa\[39\].

**Conclusion**

- Maternal Urinary tract infection is a significant factor effecting PTB, fellow by PROM and abruptio placentae.

- There is a strong association between preterm birth and oligohydramnios, gestational DM, no antenatal visit, previous preterm labor and short interval between pregnancies.

**Ethical Clearance**- Taken from The Institution’s Ethical Committee approval

**Source of Funding**- Self

**Conflict of Interest – nil**

**References**

1. Howson C.P., Kinney MV., Lawn J. March of


Factors Cause of Switching Shorter Regimen to Longer Regimen in Multidrug-Resistant/ Rifampicin-Resistant Tuberculosis Treated Patients in Dr. Soetomo Hospital Surabaya, Indonesia

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Dwi Wahyu Indrawanto¹

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Abstract

Background: Indonesia started to implement the Shorter Regimen (STR) since 2017, but not all of MDR/RR-TB patients were treated with STR until the end of treatment. The presence of side effects of one or several drugs in the STR and the resistance to fluoroquinolone and or 2nd line injection drug after starting treatment caused a switch in regimen from STR to a longer regimen. This study was conducted to evaluate the factors that caused switch STR to the longer regimen.

Methods: This was a descriptive study in MDR/RR-TB patients who received STR from October 2017 to December 2019. Patients who switch their regimen were analyzed and determine the factors cause of switching STR to a longer regimen.

Conclusion: The major cause of switching was due to the presence of resistance to fluoroquinolone and 2nd line injection drugs and incidence of prolonged QT. A diagnostic rapid test such as the line probe assay 2nd line TB drugs is absolutely a screening tool to determine MDR-TB patients, pre-XDR-TB or XDR TB as soon as the regimen is given. Monitoring and efforts to overcome prolonged QT side effects are also needed to prevent switch regimens that can affect the patient’s psychological condition.

Keyword: Shorter MDR/RR-TB Regimen, MDR-TB Longer Regimen, Switch Regimen

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is a public health crisis. Indonesia is one of 30 countries with the highest MDR or Rifampicin Resistant (RR)-TB cases in the world with 24,000 cases.¹ MDR-TB defined as TB caused by strains Mycobacterium tuberculosis that are resistant to at least isoniazid and rifampicin.² The treatment of MDR-TB is challenging. Patients with MDR/RR-TB are treated with a different combination of 2nd line drugs, usually for 18 months or more. Its long duration is associated with high cost, greater incidence of adverse reactions, and a high rate of lost-to follow up.³ Therefore, the finding of a shorter regimen (STR), more effective, lower-cost treatments for MDR-TB remains a priority. WHO updated its treatment guidelines for drug-resistant TB in May 2016 and included a recommendation on the use of the shorter MDR-TB regimen under specific conditions.³

Unfortunately, not all of STR patients were treated with STR until the end of MDR-TB treatment. The presence of drug side effects and the DST (drug
susceptibility test) results that showed resistance to fluoroquinolone and 2nd line injection drug (SLID) after the starting treatment caused a switch in regimen from STR to the longer regimen.

If phenotypic DST result shows resistance to fluoroquinolone and SLID, it must be reviewed and switched to the longer treatment regimen and also in consideration of using new drugs (bedaquiline, delamanid). Patients with QTc prolongation from baseline also concerned to decrease Mfx dose to 400 mg or may switch to drugs with a least cardio-toxic side effect.4 Programmes and their stakeholders using the standardized shorter MDR-TB regimen should intensify clinical, safety and microbiological monitoring in order to rapidly switch patients to new longer MDR-TB regimens upon first signs of non-response, ototoxicity or drug intolerance and also upon the first signs of non-response or drug intolerance.5

At present, no evidence exists on the effects of implementing the STR in the medium and long term. Information is missing on the regimen’s efficacy under programmatic conditions, the durability of its effectiveness (eg, no additional drug resistance generated), and its potential to increase numbers of patients who are ineligible for treatment because of additional resistance.6 No studies have reported the number of MDR/RR-TB patients who switched their regimen from STR to longer regimen. This study was conducted to evaluate the factors that caused switch STR to a longer regimen.

Material and Methods
A study conducted from October 2017 to December 2019 in Dr. Soetomo Hospital Surabaya, Indonesia. Based on the medical records, all RR-TB cases both new cases and previously treated cases diagnosed based on geneXpert included in this study. MDR-TB was defined as resistant to rifampicin and isoniazid. RR-TB was defined as resistant to rifampicin based on geneXpert and as the first diagnosis of MDR-TB. As most rifampin-resistant isolates are also resistant to isoniazid, rifampicin-resistant can be used as a marker for MDR M. tuberculosis7 and as the program policy for all RR-TB were directly treated with MDR regimens without waiting for the results of 1st line TB DST. Patient’s medical history, laboratory, chest X-Ray, and phenotypic DST results to the constituent drugs were analyzed to determine their eligibility for a shorter treatment regimen. Patients who switch their regimen during treatment were analyzed and determine the factors cause of switching STR to a longer regimen.

Findings
There were 224 MDR/RR-TB patients who received STR in this study with a mean age of 44.61 years old. There were 134 men and 90 women in this study as presented in table 1.

Table 1. Profile of MDR / RR-TB patients receiving STR (n=224)

<table>
<thead>
<tr>
<th>Switch regimen (n=40)</th>
<th>STR (n=184)</th>
<th>Total (n=224)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=134)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 (48%)</td>
<td>115 (62%)</td>
<td>134</td>
</tr>
<tr>
<td>Women (n=90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 (52%)</td>
<td>69 (38%)</td>
<td>90</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.05 ± 11.35 (19-63)*</td>
<td>44.73 ± 13.15 (14-74)*</td>
<td>44.61 ± 12.82 (14-74)*</td>
</tr>
</tbody>
</table>

*mean ± SD (range)

STR for MDR-TB patients consisted of 52/224 (23%) new cases and 172/224 (77%) previously treated cases. Recurrence cases were dominant with 94/224 (42%) in patients who received STR. This result was presented in table 2.
Table 2. MDR / RR-TB patients who received STR based on previous treatment history

<table>
<thead>
<tr>
<th>Previous History of TB treatment</th>
<th>Count (n=224)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic/ failures of the WHO category II regimen</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>Patients with positive smears at 3rd month of the WHO category II regimen</td>
<td>4 (1.8%)</td>
</tr>
<tr>
<td>History of using anti-TB drugs of poor or unknown quality</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>Failures of the WHO category I regimen</td>
<td>32 (14%)</td>
</tr>
<tr>
<td>Patients with positive smears at 2nd or 3rd month of the WHO category I regimen</td>
<td>12 (6%)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>94 (42%)</td>
</tr>
<tr>
<td>Returns after default</td>
<td>23 (10%)</td>
</tr>
<tr>
<td>Contact of MDR-TB cases</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>TB HIV co-infection</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>New case</td>
<td>52 (23%)</td>
</tr>
</tbody>
</table>

Of the 224 MDR/RR-TB patients who received STR, 40 (18%) were switch their regimen to longer regimen because of the presence of prolonged QT, resistance to ofloxacin (Ofl), resistance to kanamycin (Km), resistance to amikacin (Amk), adverse effect of increased serum creatinine, intolerance to Km and Cm, and adverse effect of hepatitis as presented in table 3.

Table 3. The reason for switch STR to a longer regimen

<table>
<thead>
<tr>
<th>Reason for switch</th>
<th>Count (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to Ofloxacin</td>
<td>17</td>
</tr>
<tr>
<td>Prolonged QT</td>
<td>16</td>
</tr>
<tr>
<td>Resistance to Kanamycin and or Amikacin</td>
<td>3</td>
</tr>
<tr>
<td>Adverse effect of increased serum creatinine</td>
<td>2</td>
</tr>
<tr>
<td>Intolerance to Km and Cm</td>
<td>1</td>
</tr>
<tr>
<td>Adverse effect of hepatitis</td>
<td>1</td>
</tr>
</tbody>
</table>

Of the 16 MDR/RR-TB patients who switch their regimen caused by prolonged QT, it was found that the average of potassium level was decreased, 4.06 mmol/l at the baseline test to 2.92 mmol/l when presence prolonged QT. This was presented in table 4.
Table 4. Average of potassium and natrium level in patients who switch their regimen due to prolonged QT (N=16)

<table>
<thead>
<tr>
<th>No</th>
<th>Baseline test (K and Na)</th>
<th>K and Na along with prolonged QT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K (mmol/l)</td>
<td>Na (mmol/l)</td>
</tr>
<tr>
<td>Average</td>
<td>4.06</td>
<td>135.68</td>
</tr>
</tbody>
</table>

Adverse effects of shorter regimen caused switch to longer regimen also occurred in some patients, 2/224 (0.9) switched their regimens because of increased serum creatinine and 1/224 (0.5%) because of hepatitis. Intolerance to Kanamycin and Capreomycin also caused the switch to longer regimen in 1/224 (0.5%) patient. This result suggested that allergy test before determining shorter regimen is critical to prevent the switch of regimen.

Table 5. Suspect criteria in switch STR patients caused by resistance to fluoroquinolone and or 2nd line injection (n=20)

<table>
<thead>
<tr>
<th>Suspect criteria</th>
<th>Total (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of using anti-TB drugs of poor or unknown quality</td>
<td>1</td>
</tr>
<tr>
<td>Failures of the WHO Category I regimen</td>
<td>4</td>
</tr>
<tr>
<td>Patients with positive smears at 2nd or 3rd month of the WHO category I regimen</td>
<td>3</td>
</tr>
<tr>
<td>Relapse</td>
<td>7</td>
</tr>
<tr>
<td>Returns after default</td>
<td>1</td>
</tr>
<tr>
<td>New case</td>
<td>4</td>
</tr>
</tbody>
</table>

Based on the history of previous TB treatment, of the 20 patients who switch to a longer regimen caused by the presence of resistance to Off, Km, and Amk, 4 (20%) were new cases and 16 (80%) were previously treated cases. This was presented in table 5.

Discussion

There were 224 MDR/RR-TB patients who received STR in this study, consisted of 134 men and 90 women. In Indonesia, TB is significantly more common among men than among women.8 Previously treated cases was 172/224 (77%) and new cases was 52/224 (23%) in this study. Previous anti-TB treatment was by far a strong predictor of drug resistant.9 Globally at the world level, it is estimated that there are 484,000 RR-TB cases in 2018 with a composition of 3.4%, new cases and 18% previously treated cases. In Indonesia, the percentage of new cases of MDR/ RR-TB is 2.4% and previously treated cases are 13%.1 At the Dr. Soetomo hospital since the programmatic management drug-resistant (PMDT) was implemented from 2009 to April 2019 there were 1,080 MDR/RR-TB patients with 94 (8.6%) new cases and 995 (91.4%) previously treated cases.10 Previously treated TB patients were 8.1 times more likely to develop an MDR-TB infection compared with newly diagnosed TB patients.11

MDR/RR-TB patients from recurrence cases was found higher with 94/224 (42%) in this study. Recurrence cases terminology was used due to an uncertain whether it was re-infection or relapse cases. According to several studies, recurrent TB patients are defined as patients who have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a
true relapse due to reactivation of the disease or a new episode of TB caused by a new strain of *Mycobacterium tuberculosis* or reinfection).12,13 Bacteriological relapse is defined as the reappearance of bacterial activity in a patient who has followed and completed a correct treatment and, therefore, been cured of TB. However, there can be a certainty that a patient has completed all their medication only when treatment administration is directly observed. If the treatment was not supervised, the authenticity of a relapse can be questioned. A study by Shen et al., 2017 conducted in China reported that there were 5.3% (710/13,417) of successfully treated cases had a recurrence and among 141 recurrent cases that had paired isolates, 59 (41.8%) were indicating reinfection and 82 (58.2%) were relapsed.14

Our study found 40/224 (18%) MDR/RR-TB patients who switched their regimens from shorter regimen to longer regimen. 20/224 (9%) MDR/RR-TB patients switched their regimens caused by the presence of resistance to fluoroquinolone (FQ) and or 2nd line injection drug (SLID). Resistance to Ofloxacin were 17/224 (7.6%) and resistance to Kanamycin and or Amikacin were 3 (1.3%). These patients were diagnosed with pre-XDR/XDR-TB according to drug susceptibility test (DST). Extensively drug-resistant TB (XDR-TB) defined as MDR-TB plus resistance to a fluoroquinolone and at least one 2nd line injectable agent: amikacin, kanamycin and/or capreomycin, pre-XDR-TB defined as MDR-TB plus resistance to a fluoroquinolone or one 2nd line injectable agent: amikacin, kanamycin or capreomycin.2 Pre-XDR/XDR-TB patients were not eligible to receive STR because STR was recommended by WHO only for MDR-TB patients. Of the 20 patients who switch their regimen due to resistance to FQ and or SLID, 16/20 (80%) of them were previously treated cases. WHO guideline (2016) stated that until more evidence is available, WHO recommends that the shorter MDR-TB regimen not be used in patients who have been previously treated with 2nd line drugs for more than one month or who have documented or are likely to have strains resistant to medicines in the regimen. Preferably, resistance to at least fluoroquinolones and the injectable agent used in the regimen is excluded before starting treatment by in vitro testing. In the absence of such testing, patients who are highly unlikely to be infected with resistant strains based on history of exposure, use of 2nd line medicines at country level or recent representative surveillance data may also be eligible for the shorter MDR-TB regimen.3

There were 16/224 (7%) MDR/RR-TB patients switched their regimens because of the presence of prolonged QT. Moxifloxacin (Mfx), one of drug in STR was considered to cause prolonged QT. There is evidence that Mfx is more likely to cause both QTc prolongation and a longer QTc prolongation than the other fluoroquinolones (FQs), although it is also the FQ likely to be most effective against MDR-TB. The risk of QTc prolongation with the FQs is higher when there are electrolyte abnormalities and when other QTc prolonging medications are used.15

However, the previous study reported that in adults with pulmonary TB without any cardiac risk factors, Mfx 400 mg given daily for up to 4 months did not cause an increase in the QTc interval. The study indicates that Mfx, at a dose of 400 mg given once daily, is well tolerated and without any adverse cardiac events.16 In this study, Mfx was given as national program: bodyweight <30 kg with 400 mg, 30-50 kg with 600 mg, and >50 kg with 800 mg. A study of clinical trial reported that moxifloxacin was well tolerated and not associated with increased risk of adverse reactions including prolonged QT. The study suggests that moxifloxacin could be safely used for even longer periods, though this needs to be confirmed in additional studies that are large enough to detect small but important rates of toxicity.17 A study in London also reported that Mfx is well tolerated in treating TB.18

The mean value of potassium level at the baseline test in patients who switch their regimen caused by prolonged QT was 4.06 mmol/l. This level decreased to 2.92 mmol/l along with incidence of prolonged QT. This result showed that most of patients who experienced prolonged QT were also experienced hypokalemia. Hypokalemia was defined as serum potassium level of <3.5 mmol/l. A study in France reported that hypokalemia seems to be one of the most important risk factors for QT prolongation.19

**Conclusion**

There are 40/224 (18%) MDR/RR-TB patients who switched their regimen from shorter regimen to longer regimen. The major cause of switching in this study
is due to the presence of resistance to fluoroquinolone and 2nd line injection drugs and incidence of prolonged QT with 20/224 (9%) and 16/224 (7%), respectively. A diagnostic rapid test such as the line probe assay 2nd line TB drugs is absolutely a screening tool to determine MDR-TB patients, pre-XDR-TB or XDR TB as soon as the regimen is given. Monitoring and efforts to overcome prolonged QT side effects are also needed to prevent switch regimens that can affect the patient’s psychological condition as a result of treatment, which must be started again using the longer regimen.

Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearance: Taken from the institutional ethical committee (Dr. Soetomo Hospital Surabaya).

References


Novel Combination of *Andrographis paniculata* and *Phyllanthus niruri* to Improve Performance of Laying Hens Infected with *Escherichia coli*

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Abstract

The study aims to learn the efficacy combination of *Phyllanthus niruri* and *Andrographis paniculata* to improve the performance of laying hens infected with *Escherichia coli* pathogen. Fifty laying hens of Isa Brown strain were randomly divided into 5 treatments, each treatment was divided into 10 replications (n=10). Treatment P0- (control group without infected), P0+ (hens group infected with Avian Pathogenic’s *Escherichia coli* without given extract), P1 (hens group infected with Avian Pathogenic’s *Escherichia coli* with 10% *Phyllanthus niruri* extract and 30% *Andrographis paniculata*), P2 (hens group infected with Avian Pathogenic’s *Escherichia coli* with 20% *Phyllanthus niruri* extract and 20% *Andrographis paniculata*) and P3 (hens group infected with Avian Pathogenic’s *Escherichia coli* with 30% *Phyllanthus niruri* extract and 10% *Andrographis paniculata*). Performances observed were feed consumption, Hen Day Production, eggs weight and feed conversion. Data analyzed by ANOVA and tested with the F test. The feed consumption showed in P1 was different from P3, P2, P0+ and P0-, P1 was different from P2 but not with P3, P0+, and P0- showed significantly different in each treatment. In P3 showed no differences with all treatments. The eggs weight showed different in P0+ for all treatments, while the other treatments in P1, P2, P3 and P0- showed no differences. The feed conversion showed in P0+ was different for all treatments, while other treatments showed no difference. P0+ treatment compared to (P0-, P1, P2) was significantly different, P0+ treatment compared to P3 treatment was not significantly different, between P3 and P1 treatment was not significantly different, and between P1 and P2 with P0- was not significantly different, also between P3 with P2 and P0- treatments was significantly different. Supply of *P. niruri* extract, and *A. paniculata* in laying hens can improve the performance of laying hens infected with *Escherichia coli* pathogen.

**Keywords:** *Phyllanthus niruri, Andrographis paniculata, performance of laying hens, Escherichia coli*

Introduction

The Laying hens are susceptible to colibacillosis which is caused by avian pathogenic *Escherichia coli* (APEC) as a primary or secondary agent. Colibacillosis causes growth problems, decreased production, increased number of abandoned chickens, decreased quality of carcasses and eggs, and supports the emergence of complex diseases of the respiratory, digestive and reproductive tracts which are quite difficult to treat⁴. The use of antibiotics in APEC really needs to pay attention toward the different sensitivity characteristics of *Escherichia coli* serotypes, some *Escherichia coli* serotypes are resistant to several antibiotics⁵.
Safe handling of bacterial diseases is to use medicinal plants. Indonesia as a tropical country has wealth of plants that have potential to become medicine. *Phyllanthus niruri* and *Andrographis paniculata* are plants that can be used as prevention and alternative treatment for APEC[3]. *P. niruri* is a plant that belongs to the genus *Phyllanthus* known as *P. niruri* which has antibacterial activity against APEC. *P. niruri* contains several chemical substances such as lignin, flavonoid, alkaloid, terpenoid, saponin and tannin[4]. *A. paniculata*’s constituent active compounds are lactone, tannin, saponin, alkaloid, flavonoid, and andrographolide which can increase immunity. The content of Andrographolide in *A. paniculata* can interfere the transfer pathways of viral and bacterial genetic material so it is effective against infectious agents[5].

This study aims to learn the efficacy combination of *P. niruri* and *A. paniculata* to improve the performance of laying hens (in the form of feed consumption, Hen Day Production, egg production, and feed conversion) infected with *Escherichia coli*.

**Materials and Methods**

The fifty laying hens of Isa Brown strain were randomly divided into 5 treatments, each treatment was divided into 10 replications (n=10). Treatment P0- (control group without infected), P0+ (hens group infected with Avian Pathogenic’s *Escherichia coli* without given extract), P1 (hens group infected with APEC with 10% *P. niruri* extract and 30% *A. paniculata*), P2 (hens group infected with APEC with 20% *P. niruri* extract and 20% *A. paniculata*), and P3 (hens group infected with APEC with 30% *P. niruri* extract and 10% *A. paniculata*). Performances observed were feed consumption, Hen Day Production, egg production, and feed conversion. Data analyzed by ANOVA and tested with the F-test.

**Results and Discussion**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0 (-)</td>
<td>120.80ab ± 6.52117</td>
</tr>
<tr>
<td>P0 (+)</td>
<td>118.99ab ± 1.39208</td>
</tr>
<tr>
<td>P1</td>
<td>114.05a ± 2.19062</td>
</tr>
<tr>
<td>P2</td>
<td>122.62b ± 5.35530</td>
</tr>
<tr>
<td>P3</td>
<td>118.05ab ± 4.80858</td>
</tr>
</tbody>
</table>

* Different superscripts in the same column show significant values (p <0.05)

Based on ANOVA statistical analysis, there was significant difference in feed consumption (p <0.05), then continued with Duncan test with a significant level of 5% to compare the differences obtained in other treatments. The results of Duncan test showed that P1 was different from P3, P2, P0+ and P0-, P1 was different from P2 but not with P3, P0+, and P0- showed significantly different in each treatment. P3 showed no differences with all treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0-</td>
<td>61.81b ± 2.79</td>
</tr>
<tr>
<td>P0+</td>
<td>57.22a ± 0.76</td>
</tr>
<tr>
<td>P1</td>
<td>61.05b± 3.05</td>
</tr>
<tr>
<td>P2</td>
<td>64.04b ± 2.34</td>
</tr>
<tr>
<td>P3</td>
<td>63.20a ± 2.26</td>
</tr>
</tbody>
</table>

*Note: a,b,c,d,e Different superscripts in the one column show significant values (p <0.05)*
Based on ANOVA statistical analysis, there was significant difference in feed consumption ($p < 0.05$), then continued with Duncan test with a significant level of 5% to compare the differences obtained in other treatments. The results of Duncan test showed different in P0+ for all treatments, while the other treatments in P1, P2, P3 and P0- showed no differences.

### Table 3. Mean of Hen Day Production and Standard Deviation in Layer Hens Infected by *Escherichia coli* with *Phyllanthus niruri* and *Androgaphis paniculata* Extracts Therapy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Hen Day Production (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0-</td>
<td>98.25b ± 3.5</td>
</tr>
<tr>
<td>P0+</td>
<td>87.75a ± 3.5</td>
</tr>
<tr>
<td>P1</td>
<td>94.75ab ± 3.5</td>
</tr>
<tr>
<td>P2</td>
<td>96.50b ± 4.04</td>
</tr>
<tr>
<td>P3</td>
<td>91.25ab ± 6.70</td>
</tr>
</tbody>
</table>

*Different superscripts in the same column show significant values ($p < 0.05$).

Based on ANOVA statistical analysis, there was significant difference in feed consumption ($p < 0.05$), then continued with Duncan test with a significant level of 5% to compare the differences obtained in other treatments. The results of Duncan test showed P0+ was different from P0- and P2 was not different from P1 and P3. P0- was different from P0+ and showed no differences with P1, P2, P3. P1 showed no differences with all treatments. P2 was different from P0+ and showed no difference with the other treatments. P3 showed no differences with all treatments.

### Tabel 4. Mean of Feed Conversion and Standard Deviation in Layer Hens Infected by *Escherichia coli* with *Phyllanthus niruri* and *Androgaphis paniculata* Extracts Therapy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0-</td>
<td>1.95a ± 0.027</td>
</tr>
<tr>
<td>P0+</td>
<td>2.07b ± 0.034</td>
</tr>
<tr>
<td>P1</td>
<td>1.87a ± 0.055</td>
</tr>
<tr>
<td>P2</td>
<td>1.91a ± 0.056</td>
</tr>
<tr>
<td>P3</td>
<td>1.87a ± 0.067</td>
</tr>
</tbody>
</table>

*Note: a,b,c,d Different superscripts in the one column show significant values ($p < 0.05$).
Based on ANOVA statistical analysis, there was significant difference in feed consumption ($p < 0.05$), then continued with Duncan test with a significant level of 5% to compare the differences obtained in other treatments. The results of Duncan test showed P0+ was different for all treatments, while other treatments showed no difference.

*P. niruri* and *A. paniculata* contains tannin, flavonoid, saponin dan alkaloid compounds[6]. *P. niruri* contains terpenoid, flavonoid, alkaloid, saponin and tannin compounds. According to Gunawan immune cells to increase the immune system[7]. Flavonoids inhibit the function of cell membrane by interfering with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not formed completely which causes cell death[8]. Alkaloids in *P. niruri* are alkaline compounds containing nitrogen atoms that function as antimicrobial, antimalarial, antiarrheal and antidiabetic. Alkaloilds work by destroying the peptidoglycan constituent components in the bacterial cell wall and inhibiting the synthesis of nucleic acids, thereby inhibiting the energy metabolism of bacterial cells[9]. Saponins are antimicrobial, these compounds can reduce the surface tension of the cell walls which causes the cell walls to lysis and eventually bacterial death[10]. Tannin compounds, which are compounds of *P. niruri*, have mechanism of action to inhibit and kill bacteria that react with bacterial cell membrane and destroy or inactivate the function of genetic material in bacterial cells[11]. The mechanism of tannin compounds is that they enter into the cell walls of bacteria that have been lysed due to the action of saponin and flavonoid compounds so tannin compounds can easily enter the bacterial cell wall and coagulate the protoplasm of bacterial cells. Tannins also have target on cell wall polypeptides so the formation of the cell walls becomes imperfect which causes bacterial cells to become lysed due to osmotic or physical pressure, so the bacterial cells will die. The improvement in egg production is also influenced by terpenoids which act as antibacterial inhibitors of *E. coli*[12]. *A. paniculata* has an active component, namely andrographolide which has an antibacterial effect against various microbes by damaging the bacterial cell membrane resulting in inhibition of specific enzyme biosynthesis and enhancing the immune system[13]. Tannins are included polyphenol compounds that can inhibit bacterial cell adhesion, inhibit enzymes and disrupt protein transport in the cell layer so bacterial cells become lysed due to osmotic pressure and physical pressure, while flavonoid compounds can inhibit bacterial growth by damaging the arrangement of the plasma membrane and cause changes in the permeability of the bacterial cell wall at low concentrations[14]. Saponins have antibacterial mechanism by reducing the surface tension of the bacterial cell walls so that they interfere with the survival of the bacteria. Alkaloid compounds function as antibacterials and have mechanism of destroying the components of peptidoglycan in bacterial cells so the bacterial cell wall is not formed completely and causes cell death[15]. Supply of *A. paniculata* and *P. niruri* extracts combination causes healing in layer hens infected with APEC and results in returning to the normal process of egg formation and egg production. Supply of combination dose 20%:20% of *P. niruri* and *A. paniculata* extracts improves the performance of laying hens.

On the other hand, Indonesia is an archipelago with approximately 17,508 islands and is covered by tropical rain forest, seasonal forest, swamp, subalpine shrub vegetation, coastal vegetation, and mountain vegetation. With its reflective mixture of Asian and Australian native species, Indonesia is stated to possess the second largest biodiversity in the world, with around 40,000 endemic plant species including 6,000 medicinal plants. Consequently, Indonesia is rich in medicinal plants which were used by its population traditionally from generation to generation in curing. Therefore, natural resources in Indonesia are very supportive for the use of herbal medicine-based therapies in in vitro or in vivo tests[16,17,18,19,20].

**Conclusion**

In conclusion, from the result of this study, it can be concluded that *P. niruri* and *A. paniculata* combination improves the performance of laying hens infected with *E. coli*.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Source of Funding:** This study supported by the Ditjen DIKTI (Directorat General of Higher Education) for funding this study through scheme Program Penelitian Unggulan Perguruan Tinggi (PTUPT/Universities
Leading Research Program Decentralization).

Acknowledgements: The authors are thankful to the Rector of Universitas Airlangga and Director of Research and Innovation Universitas Airlangga, Indonesia for facilitating this research and we additionally thank Arif Nur Muhammad Ansori for help in editing the manuscript.

Ethical Approval: This study was approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

References


Antibacterial Activity of Extract Ethanol Bidara Leaves 
(Ziziphus spina-Christi L) on Enteropathogenic coli

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Abstract
Prokaryotic bacteria are microorganisms that can be beneficial as normal flora, but also can have health due to disease pathogens and is for the host. The purpose of this study is to find compounds in extract ethanol bidara leaves those compounds and activities against bacteria Enteropathogenic coli. This study shows the flavonoid compounds and tannin in extract ethanol bidara leaves (Ziziphus spina-Christi L) to minimum inhibitory zone at concentrations 50% and minimum bactericidal at concentrations 75%. The higher concentration extract ethanol Bidara leaves the larger the drag zone produced. The research is experimental study looking the extract ethanol antibacterial activity Bidara leaves with the methods in vitro test diffusion. This study found a chemical compound which is found in extract ethanol Bidara leaves (Ziziphus spina Christi L) is flavonoid and tannin have antibacterial activity10⁶ CFU/ml against bacteria Enteropathogenic coli to minimum inhibitory zone at concentrations 50% and minimum bactericidal at concentrations 75%.

Keywords: antibacterial, bidara leaves (Ziziphus spina-Christi L), EPEC, MIC, MBC

Introduction
Prokaryotic bacteria are microorganisms that can be beneficial to health as normal flora, but it could also impact because it is detrimental to the host pathogens and cause disease¹.

Antibacterial is a substance that can interfere with growth or kill bacteria by the mechanism of disturbing the metabolism of bacteria. An antibacterial ideal having been selective toxicity, antibacterial substance that was only harmful to bacteria causing infections but are not dangerous for host Hospes or body².

Antibacterial substance is compounded capable of inhibiting the growth of microbes and can be used in the treatment of in humans, animals and plants. Based on the nature of selective toxicity, antibacterial divided into two, bakteriostatic is working in a way inhibits the multiplication of bacteria and which is a bactericidal which kills bacteria. Bakteriostatik can act as a bactericide in high concentration³. An antibiotic among bactericide among them are penicillin, cephalosporin, aminoglikosida, kotrimoksazol, and isoniazid rifampisin. While among those targets are a sulfonamide bakteriostatik, tetracycline, kloramfenikol, erytromisin, trimetropin, linkomisin, klindamisin peraminosilat and acid².

Treatment of diseases caused by bacteria could be done with the purpose of hinder/kill pathogenic bacteria infecting humans in synthetic antibiotic. But research finds treatment with antibiotics this risk having synthetic resistant so as to cause treatment failure and patients being infected for a long time⁴. When viewed from risk of antibiotic resistance to bacteria, synthetic pathogenic it takes to find alternative solution medicine safe, cheap, easily obtained a new drug and better as a substitute for
synthetic antibiotic. Of compound substances can be obtained antibacterial of microbes, herbs and animals. The opportunity to obtain antibacterial natural material in Indonesia very large, considering that Indonesia is a rich in biodiversity\textsuperscript{5}. The use of traditional medicines in Indonesia is substantially is part of the Indonesian nation, culture this will require the existence of the development and research new drugs are derived from plants. The advantage of the use of traditional medicines is easy to get around us and empirically traditional medicine’s ability to heal various kinds of diseases, but efficacy and their ability not proven clinically\textsuperscript{6,7}.

Antibacterial activity can be measured by in vitro methods to determine the antibacterial a substance in solution and sensitivity to concentration presented by a bacteria. Bacterial sensitivity to an antibacterial can be tested by diffusion and dilution methods. The diffusion method is an antibacterial activity test that is often used because it’s relatively easy, affordable, a stage of work does not require special skills and the result are obtained faster\textsuperscript{8}.

Bidara (\textit{Ziziphus spina-Christi L}) has been proven could heal some diseases as indigestion, weakness, complaints, hearts obesity, bladder problems, diabetes, infections of the skin, lost appetite, diarrhea, fever, insomnia, as a tranquilizer and cancer\textsuperscript{9,10}. In Saudi Arabia, this plant has been used for the treatment of various diseases such as indigestion, obesity, their complaints, infections of the skin, fever, diarrhea, bronchitis, diabetes, anemia and insomnia\textsuperscript{11}.

The bidder is an evergreen tree that grows wild throughout the islands of Java, Bali, Madura and Sumbawa (Nusa Tengara Barat) at an altitude below 400 meters above sea level. All parts of bidara are used in traditional medicine (leaves, fruit, seeds, roots, and stems) now supported by several research found plant bidara contain the alkaloid, glycoside, tannin, flavonoid, quinone, saponin and steroid/terpenoid\textsuperscript{12,13,14}. Other research also found that stems, leaves and seeds bidara having antibacterial activity, anti-inflammatory, anti-fungal, anti-cancer by in vitro and in in vivo\textsuperscript{15,16,17,18}.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Filtering of phytochemistry & Leave & Fruit & Seed \\
\hline
Alkaloid & + & + & - \\
Flavonoid & + & + & + \\
Saponin & + & + & - \\
Tannin & + & + & + \\
Kuinon & + & + & + \\
Steroid/Triterpenoid & + & + & - \\
\hline
\end{tabular}
\caption{The compound in Bidara}
\end{table}

To the best of our knowledge, there have been no reports on the effects of the species on the antibacterial activities of the \textit{Ziziphus spina-christi L}. Thus, the aim of the present study was to i) estimate the levels of total tannin compounds and total flavonoids of leaves, ii) study the antibacterial activities of \textit{Ziziphus spina-Christi L} leaves to \textit{Enteropathogenic coli} $10^6$ CFU/ml \textit{in vitro}.

**Materials and Methods**

**Site of the study:** The study was carried out at the biochemistry laboratory, Faculty of Medicine, University of Airlanga, Surabaya.

The design of this study was an experimental study by looking at the antibacterial activity test result of bidara leaf ethanol extract carried out by the diffusion test method in vitro. Diffusion test using \textit{disk diffusion} as a medium for ethanol extract of bidara leaves using
EPEC $10^6$ CFU/ml as a test bacterium. The required data is the diameter of the inhibitory zone formed on the MHA media when conducting the sensitivity test of the disk diffusion method.

**Sample collection and Preparation**

Bidara leaf extract is made from 3 kg of fresh bidara leaves harvested from Madura Island-East Java, cleaned and dried leaves at the temperature of an oven 70°C to dry. Simplicia dried bidara leaves are blended and sieved using 40 mesh sieve to form 560 g of powder, powder obtained used for making extract ethanol bidara leaves. Next the process maceration simplicia Bidara leaves by soaking 560g of Bidara leaves with 2.300 ml ethanol 96 % and left be closed for three days and placed in a sheltered from direct sunlight. During the soaking, marinade stirred several times with the purpose of improving the effectiveness of the process diffusion compound dissolved in a search liquid. Simplicia and the search fluid are filtered and squeezed to get the first macerate liquid. The pulp is soaked again with 750 ml ethanol for three days to get the second macerate. The second macerate then combined with the first macerate. Macerate obtained settled for 1x24 hours and deposited. Macerate concentrated using an evaporator rotary at a temperature 50°C order to obtain extract viscous bidara leaves free from a solvent. Next included to Erlenmeyer plus a solvent ethanol 96 % and whipped 2-3 hours. Next undergone a filtering Phytochemistry the flavonoid and tannin.

**Making Suspense Bacteria Test**

1 ml of *E. coli* stock cultured on nutrient agar (NA) media at temperatures 37°C for 24 hours. Then will be the manufacture of suspension bacteria culture test by taking *E. coli* and dissolved in solution copy (0.9% NaCl) in aseptic in the tube different, each 5 ml solution NaCl 0.9%. A suspension that is formed equalized with a standard McFarland No. 0.5 that is 1.5 x 108 CFU/ml. For the manufacture of the media MHA (*Muller Hinton Agar*) to do with how to weigh 9,5 g Muller Hinton Agar/MHA (38 g/L) with a composition medium (Beef infusion 300 g, Casamino acid 17,5 g, Starch 1,5 g) dissolved in 250 ml aquades then heated to boiling then sterilized in an autoclave for 20 minutes with the air pressure 1 ATM temperature 121°C.

**In Vitro Antibacterial Study**

The antibacterial activity undertaken using pathogenic bacteria *Escherichia coli* (EPEC) in aseptic with the methods disc diffusion. Suspension bacteria test spread evenly in a media MHA by using a swab, sterile cotton, then settled for a few minutes until suspension bacteria percolate in a media. A next paper strain that serves as the accommodates extract Bidara leaves lay on plate agar and incubated at a temperature 37°C among 24 hours. The result of the observation obtained of the whereabouts of zone clear formed all over paper discs which indicates the presence of zone obstruct in the growth of bacteria. The greater cleared zone, the large the ability obstruct extract ethanol Bidara leaves against bacteria. Zone category obstruct as on a table 2.

<table>
<thead>
<tr>
<th>No</th>
<th>Diameter (mm)</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diameter &gt; 12mm</td>
<td>Strong (+++)</td>
</tr>
<tr>
<td>2</td>
<td>Diameter 9&lt;Ø≤12mm</td>
<td>Moderate (+)</td>
</tr>
<tr>
<td>3</td>
<td>Diameter 7&lt;Ø ≤9mm</td>
<td>Weak (+)</td>
</tr>
<tr>
<td>4</td>
<td>Diameter = 6mm</td>
<td>No obstacles (-)</td>
</tr>
</tbody>
</table>
Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The determination of minimum levels of the barriers do with the antibacterial activity leaves extract ethanol Bidara diffusion tests with a method of in vitro culture with bacteria to the media and be counted in a paper disc with bacteria test Enteropathogenic coli $10^6$ CFU/ml. The determination of minimum levels of kill characterized by the whereabouts bacterial growth in a media culture (nutrient agar) by looking at the number of colonies of every series extract. Concentration The colony incubating expressed by growing or do not grow.

Data Analysis

Data analysis was conducted in descriptive having acquired an obstruent minimum data concentration and kill minimum concentration extract ethanol leaves Bidara against bacteria EPEC $10^6$ CFU/ml. The results of the study and compared with parameters and identified as an obstruent kill zone and the ability to see minimum antibacterial activity Bidara leaves extract ethanol as an antibacterial against Eschericia coli.

Results

The Results Of The Phytochemistry Extract Ethanol Bidara Leaves (Ziziphus spina-Christi L).

The Phytochemistry conducted in this research is the flavonoid tannin and. Tannin, on the 0.1 g extract ethanol leaves bidara added 5 ml aquades then simmer for a few minutes. Filtrat strained and added FeCl$_3$ 1%. Change colors become a deep blue color or black, greenish formed show positive results in the compound tannin extract leaves bidara tested. In the flavonoid, as many as 5 mg extract leaves bidara dissolved in 5ml hot water, simmer for 5 minutes, and strained. Filtrate obtained them added MG powder, 1ml of concentrated sulfuric acid plus 2ml ethanol. Beaten and let separate strong. Formed red, yellow or to the ethanol, orange showed flavonoid compounds

Table 3. Test Phytochemistry Extract Ethanol Bidara Leaves (Ziziphus spina-Christi L)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid (mg/100g)</td>
<td>65,83</td>
</tr>
<tr>
<td>Tannins (µg/g)</td>
<td>179,67</td>
</tr>
</tbody>
</table>

Table on shows that an extract ethanol Bidara leaves (Ziziphus spina-christi L) containing flavonoid as much as 65,83 mg/100g and taninns179,67 µg/g.

Result Test of Obstruent Zone

Extract ethanol Bidara leaves based on the measurement result after passing the incubation period of 24 hours and formed meaningful obstruent zone that bacteria unable to grow or develop due to the influence of antibacterial substance given, shown in table 4.

Table 4. Measurement Zone Obstruent Extract Ethanol Bidara Leaves (Ziziphus spina-Christi L) against Enteropathogenic coli $10^6$ CFU/ml on Various Concentration

<table>
<thead>
<tr>
<th>Concentration Extract Ethanol Bidara Eaves</th>
<th>Obstruent Zone</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>7 mm</td>
<td>Weak</td>
</tr>
<tr>
<td>75%</td>
<td>11 mm</td>
<td>Moderate</td>
</tr>
<tr>
<td>100%</td>
<td>11,1 mm</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
Extract ethanol Bidara leaves could impede the bacterium Escherichia coli, with a diameter of 11,1 mm at 100% concentration, 75% concentration with a diameter of 11 mm and 25% concentration with a diameter of 7 mm. From the research is found in the bacterium *Escherichia coli*, each concentration extract ethanol leaves bidara have differences in Obstruent Zone. At concentrations extract higher having a zone obstruent larger compared by an astronaut with zones around paper disk with low extract lower. This proved that the higher concentration extract ethanol leaves bidara the more high zone obstruent produced.

**Image 1. Antibacterial Activity Extract Ethanol Bidara Leaves a Method of Paper Discs to Escherichia coli.**

The picture above shows the diameter of obstruent sub extract ethanol Bidara leaves (*Ziziphus spina-Christi* L) against *Enteropathogenic coli* $10^6$ CFU/ml on various concentrations.

**Table 5. The Minimum Bactericidal Concentration The Extract Ethanol Bidara Leaves (*Ziziphus spina Christi* L) against *Enteropathogenic coli* $10^6$ CFU/ml on Various Concentration**

<table>
<thead>
<tr>
<th>Extract Ethanol Bidara Leaves Concentration</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>75%</td>
<td>-</td>
</tr>
<tr>
<td>50%</td>
<td>+</td>
</tr>
<tr>
<td>25%</td>
<td>+</td>
</tr>
<tr>
<td>12.5%</td>
<td>+</td>
</tr>
<tr>
<td>6.25%</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: + = growth; - = not growth

Table 5 showed Minimum Bactericidal Concentration had been incubated for 18-24 hours at temperature 37°C. Based on the results of the antibacterial activity extract ethanol leaves bidara against *Enteropathogenic coli* the results concentration of 75% and 100% there is no bacterial growth, on the concentration 6,25% to 50% is the growth of bacteria.

**Discussion**

The extract ethanol leaves bidara have diameter obstruent of different suits distinction concentration. The greater the concentration of the larger the diameter obstruent that the establishment, in order to know the concentration and diameter obstruent is directly proportional each other. In table 4 it is evident that concentration 50% extract ethanol leaves bidara obtained diameter obstruent zone of 7 mm, 75% concentration of 11 mm and concentration of 100% 11,1 mm, it can be said that the higher concentration extract used the more diameter obstruent formed zone. These facts in accordance with statements from Pelczar and Chan$^{22}$ that the higher concentration antibiotic substances the more a fast growth microorganisms killed and impeded.

In the levels of kill minimum found that bacteria *Escherichia coli* can not grow in a mediaNA given extract ethanol leaves bidara concentration 75% dan 100%. This indicates that extracts ethanol leaves bidara have the ability kill bacteria *Escherichia coli* at concentrations 75% dan 100% but has no power killed at concentrations 50%, 25%, 12.5% dan 6.25%. The results of the study is based on research conducted Edy *et al*$^{15}$ who discovered the methanol extract bidara could hinder growth some pathogenic bacteria.

From the observation of MIC and MBC show positive results, this is caused by the existence of a metabolite secondary in extract ethanol leaves bidara so as to give the effect on the growth of bacteria test. Working mechanism substance flavonoid as an antibacterial is form a compound complex with a protein extracellular and dissolved order to be able to destructive the cell membrane bacteria that followed release of intracellular compound$^{23,24,25}$. According to Cushnie and Lamb$^{26}$, apart from its role in inhibitory in DNA – RNAsynthesis by intercalation or hydrogen bonds with the accumulation of a nucleic acid, flavonoid would play a role in resisting energy metabolism. Compound with protein through flavonoid locks down into hydrogen bonds resulting in protein structure being broken, instability and the cytoplasm of a cell wall destroyed. Causing integrity destroyed cytoplasm makes macromolecules of ions and the lost its shape and become lysis$^{27}$. 


Tannin have antibacterial activity associated with its ability to activate adhesion the microbes also activation enzyme, and interfere with the transport protein in bacterial cells. According to Sari, the tannin will have targets in polypeptide the wall of the cells so that the formation of the cell walls into less than perfect that causes bacterial cells be lysis because of the pressures of osmotic and physical and the bacteria would die. Microorganisms growing under conditions aerobic need iron for various function, including reduction from a precursor ribonukleotida DNA. This is caused by capacity a fastener solid steel by tannin. Working mechanism tannin as an antibacterial is by means hinder an enzyme reverse reverse transcriptase and DNA topoisomerase and the bacteria from being can be formed. According to Nahak, compound mechanism tannin show antibacterial proline bonded with activity in which are rich in protein leakage and damage occurs bacterium cell wall and lead to the death of the bacteria. At low concentration phenol work with destructive membrane cytoplasm and can cause to leak the cells. While in large concentration the substance coagulated with protein cellular, activation is very effective when bacteria in the division, where the phospholipid around cells are on the very thin and phenol penetration and can easily destroy cells.

**Conclusion**

The research is from a chemical compound which is found in extract ethanol left bidara (Ziziphus spina Christi L) is flavonoid and tannin have antibacterial activity as against Enteropathogenic coli with zones inhibitory was at concentrations 75 % and 100% and minimum bactericidal at concentration 75%. The higher concentration extract ethanol leaves bidara, the greater the obstruent and the kill against bacteria Enteropathogenic Coli.

**Suggestion**

Necessary other experiment by changing concentration treatment to find concentrations of MIC and MBC more effective and antibiotic as comparing and control.

**Acknowledgements:** The author would like to thank Prof. Dr. Merryana Adriani, S.Km., M. Kes and Prof. R. Bambang Wirjadmani, M.S., MCN., Ph.D., Sp. GK has helped and supported in this research and all parties involved in this research.

**Conflict of Interest:** The author states that there is no conflict of interest regarding the publication of this article.

**Source of Funding:** Personal researcher.

**Ethical Clearance:** This study was approved by Health Research Ethics Committee, number 218/EA/KEPK/2019, Faculty of Public Health, University of Airlangga, Surabaya.

**References**


The Chemical and Physical Parameters as Indicator of Office Air Quality at PT X Coal Mining Company

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Abstract

**Background:** Coal still ranks the second largest source of total global energy demand, which is complemented by the high-risk nature of coal mining activity. Therefore, it is important to discuss the health issues of coal workers. One of the risks associated with coal mining activities is poor indoor air quality (IAQ) due to the high concentration of airborne pollutants. **Methods:** This study aims to evaluate the results of air quality measurements in the PT X office, and the method used was a descriptive approach with quantitative secondary data. Furthermore, the measured variables are NO2, SO2, CO2, CO, Pb, PM10, temperature, humidity, and noise. **Conclusion:** The results showed that there was an IAQ problem, namely noise, temperature, and humidity which exceed the recommendations. Therefore, the IAQ management implemented by PT X was still systematically and comprehensively developing.

**Key Words:** Coal mining, office air quality, chemical parameter, physical parameter.

Introduction

Coal mines air are well known for its air pollutant namely sulfur dioxide (SO2), nitrogen dioxide (NO2), VOC (volatile organic compound), PM10, and PM2.5. Various health impacts arise due to the risks contained in mining air, one of which is pneumoconiosis, which includes both black lung and silicosis. Meanwhile, in America, it was noted that black lung is the latent killer for 4,118 mining workers from 2007-2016. Significance of the problem of air health risks in the coal mine is greater in the indoor and outdoor work areas. However, the number of indoor air pollutants is 2-5x greater than outdoor air. In addition, it was reported in the late ‘80s that humans spent 90% of their time indoors. As a result, people that work in offices, as well as perform their main work indoors are at the highest risk of being exposed to air pollutants.

These pollutants cause poor indoor air quality and also have various impacts, which include, affecting the cognitive performance of workers and subclinical disturbances that leads to a decrease in work productivity. Furthermore, health problems such as eye irritation, headaches, and allergies, as well as discomfort effect results in high absenteeism.

In addition to disrupting cognitive performance, worker productivity, and a high rate of absenteeism, poor air quality causes the ‘typical’ disease of this problem, which includes, SBS (Sick Building Syndrome) and BRI (Building Related Illness). SBS is a term used to describe a situation where the occupants of a building experience acute health problems and discomfort associated with the length of time spent in the building, but no disease was identified and the complaint disappears once someone leaves the building. Meanwhile, BRI is a condition in

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which symptoms of health problems are diagnosed and are known to be related to indoor air contaminants. PT X is inseparable from the health risks caused by poor indoor air quality. In an office located in the middle of the mine site, it is possible to infiltrate mine air into the room. PT X performs four main business processes, namely mining operations, hauling operations, coal processing, and barge loading (CPBL) Kelanis, as well as supporting facilities. Meanwhile, there are more than 155 contractors who work to assist PT X in its business processes in Kalimantan (Borneo). Therefore, the discussion of managing health risk control due to indoor air quality in PT X’s coal mine needs attention.

**Materials and Method**

The method used was a quantitative data with a descriptive approach to evaluate the results of air quality measurement in the office rooms at the PT X coal mining location. Furthermore, the data were collected by reviewing secondary data in the form of documents on the results of air measurements for the work environment at the Kalimantan office PT X site in 2019 and primary data in the form of interviews with informants, such as OHS managers and environmental staff. The variables studied consisted of two parameters, namely chemical parameters such as NO2, SO2, CO, CO2, Pb, and PM10, as well as physical parameters such as noise, temperature, and humidity. Secondary data for the measurement of chemical and physical parameters at the PT X offices were collected from July-December 2019.

**Result and Discussion**

All the rooms measured have the similarity, namely their main function as office room to manage administration. However, the characteristics of each building and room are varies depending on its location and the presence of other factors that affect the IAQ. The SM, Plant and Main Office are located in the SEG III work area, namely in the CPBL (Coal Processing & Barge Loading) area, which is located close to the coal crushing work process as well as in the stockpiling and loading of coal into the barge. The Main Office is a building that is the center of administrative activities at SEG III and uses split AC. There is an Office Plant not far from the main office, which also has administrative activities and is very close to the equipment maintenance process that involves welding. In addition, the Office Plant is also located not far from the landfill (TPS) and uses split AC. Office SM has similar characteristics to other rooms in the SEG III work area.

The KM 69 Office, Mine Office, and the MIA Logistics Office on the first floor are located in the SEG II work area, namely hauling or coal transportation routes using trucks/trailers. Furthermore, the KM 69 Office room serves as a checkpoint for the 80 km coal haulage route. Therefore, there is a parking area around this building that can accommodate up to 10-15 trailers. This building is located in the middle of a tree-free field which consists of two floors with a generator, landfill (TPS), and a smoking area. The characteristics of Mine Office are similar to KM 69 Office, because of its location in the middle of a transportation route, which is a field without trees. However, this building is made of a container that has only one room with an air conditioner without ventilation. In addition, this room is only for administrative activities and is used by officers responsible for transporting coal. The logistic office on the first floor of the MIA is located close to a heavy equipment repair workshop, and the building only has one room with administrative activities as well as spare parts inside and outside. Also, the type of air conditioner used is split.

The result shows that the highest NO2 concentration is in the Main Office room at 0.01 ppm, but this result is still below the TLV of Permenaker No. 5 of 2018. The highest SO2 concentration is in the Office Plant room at 0.03 mg/m3, and the highest CO concentration in the KM 69 Office room is 8 ppm, which is close to the 10 ppm requirement regulated in Permenkes No. 48 of 2016. Furthermore, the highest Pb concentration is 1.3 µg/m3 at the main office, and the highest PM10 concentration is reported in the Office Plant room with 0.016 mg/m3, while the highest CO2 concentration in the Mine Office room is 327 ppm, but these two values are still below the requirements of Permenkes No. 48 of 2016.

The measurement results of physical factor variables reported that several variables exceed the requirements of Permenkes No. 48 of 2016. The temperature variables in all rooms, namely Office SM is at 31.6°C, Plant at 31.9°C, Main Office at 29.8°C, KM 69 is at 29.1°C, Mine Office is at 29.6°C, and the MIA 1st Floor Logistics
Office is at 28.4°C, which exceeds the requirements of Permenkes No. 48 of 2016, namely 23-26°C. The humidity problems only occur in the Main Office by 61-67%, exceeding the requirements of 40-60%. Also, the highest noise occurs in the MIA 1st Floor Logistics Office by 65.8 dBA, which exceeds the standard of 55-65 dBA for office activities.

The measurement results for Office SM of 31.6°C and Plant of 31.9°C were included in the hot category. Furthermore, the measurement results for the Main Office at 29.8°C, Office KM 69 at 29.1°C, Mine Office at 29.8°C, and the MIA 1st Floor Logistics Office at 29.8°C are included in the warm category. This heat source is estimated to be derived from the characteristics of all measured buildings located in the middle of the mine area with few or no trees. Therefore, there is a need to identify the source of the problem in further research, such as AC.

Offices that are too hot causes residents to feel tired. Therefore, it is necessary to take corrective measures to control the IAQ issue related to temperature to achieve thermal comfort which is important for worker productivity. Thermal comfort is a condition where a person wears normal clothes without feeling too cold or too hot, and it is important to a person’s well-being and productivity. Although everyone’s temperature preferences are different, the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 55 - 2013 recommends a temperature of 23-26°C, with thermal Environmental Conditions for Human Occupancy that satisfies 80% of building occupants.

The 61-67% humidity in the Main Office room is categorized as high because it exceeds the requirements. The estimated source is the use of the building which is the center of administrative activity in SEG III. Also, there is the possibility of workers to enter and exit the hot mine area in sweaty conditions. However, the identification of a source with high humidity content needs to be conducted more deeply in further research. High humidity makes the room stuffy, increases the perception of heat felt in the room, and supports the development of microorganisms. Therefore, the humidity of the Main Office also needs to be controlled because humidity with temperature (air and radians), clothing, and body metabolic rate are factors that affect thermal comfort.

The results of noise measurements at the MIA Logistics Office on the 1st floor show a value of 65.8 dBA which exceeds the Permenkes No. 48 of 2016. The source of this noise may be from an office located near a heavy equipment repair workshop. Furthermore, the impact caused by noise that exceeds the requirements in the office is distracting concentration at work because of divided auditory attention. This disruption in concentration also causes an increase in annoyance which in the long term will result to stress.

Office KM 69 which shows the highest CO measurement results has a trailer parking area, a smoking area, and a generator. The existence of these facilities and equipment is thought to be the source of the high CO concentration in this room. Although the CO at Office Km 69 does not reach the highest recommended concentration, the exposure of low CO concentration causes fatigue and heaviness in the chest for people with heart disease, while the impact on moderate concentration exposure is angina, reduced vision ability, and reduced brainpower.

Townsend, Robert, and Maynard reported that CO exposure of 9 ppm causes the formation of 2.5% COHb (carboxyhemoglobin) in the blood. A total of 2-20% COHb content in the blood causes subtle effects on visual perception, hearing, motor and sensorimotor performance, alertness, and other measures of neurobehavioral performance. Therefore, the measurement result in the KM 69 Office room of 8 ppm is already a number that needs to be considered and controlled.

**Conclusion**

The measurement of air quality in PT X offices shows the result of the evaluation results of several variables that exceed the standard. Furthermore, the variables that exceed the standard, however, are based solely on the physical parameters, such as noise, temperature, and humidity. The temperature that exceeds the requirements is reported in the six examined rooms. Meanwhile, humidity that exceeds the requirements is only in one room, namely the Main Office. Also, noise that exceeds the requirements only occurs in the MIA 1st
floor Logistics Office. Despite being in the middle of a coal mining process area, there are no chemical factors that exceed the standard.

Ethical Clearance – The study protocol was approved by The Research and Community Engagement Ethical Committee, Faculty of Public Health, Universitas Indonesia with approval letter number Ket-314/UN2.F10.D11/PPM.00.02/2020.

Source of Funding- International Indexed Publication for Health, Science, and Technology Grant Universitas Indonesia, Contract No: NKB-2377/UN2.RST/HKP.05.00/2020

Conflict of Interest - Nil

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The Relation between Exercise Duration and Intensity on Phosphocreatine (PCr) Level: an Article Review

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Abstract

Exercise is a physical activity that planned, structured, and sustainable. Exercise has 4 criteria, that is frequency, intensity, type, and duration. During exercise, phosphocreatine (PCr) depletion increases, and early intracellular acidosis has occurred. These changes contribute to a decrease in training capacity in terms of training duration. This literature review aimed to determine the relationship between the intensity and duration of exercise with the concentration of PCr and recovery factors. The literature study has carried out by selecting an Experiment research design. The results of the review showed that PCr was the largest energy contributor in the first 10 seconds of exercise in the heavy-intensity exercise where phosphocreatine triggers energy without oxygen or anaerobic. The recovery of PCr influenced by a person’s health condition and age. The concentration of PCr in children was higher than in adults because of the relatively high rate of oxidative ATP formation. It proved that there was a significant relationship between PCr and the duration and intensity of exercise.

Keywords: Exercise duration, Exercise intensity, PCr, Phosphocreatine

Introduction

Health is considerable for humans. Everyone must be healthy to maintain physical fitness so that they can carry out daily activities. According to the Indonesian Law No. 23 of 1992 concerning health states that health is a state of well-being of body, soul, and society which enables everyone to live productively socially and economically(¹). Health can be pursued in various ways, one of which is by exercising.

Exercise is a physical activity that planned, structured, and continuous by involving regular and repeated body movements to improve physical fitness and achievement(²). Exercise is useful for maintaining and increasing mobility and independence to move in human bio-psycho-sociologic life(³). Exercise has four criteria, that is frequency, intensity, type, and duration(⁴).

Exercise can stimulate the disruption of homeostasis and change the physical and chemical environment of cells. Exercise can cause body temperature to increase, increase blood acidity, decrease oxygen in fluids, and increase CO2. Environmental changes in the body start at the receptors, namely body cells that will stimulate complex response pathways. This pathway causes changes in nerve activity (nerve pathways), hormonal changes (hormonal pathways), and exchanges in specific pathways (intrinsic pathways). Also, chemical, mechanical and thermal stimuli affect changes in metabolic, cardiovascular, and ventilator functions to meet increased demand(⁵).

Exercises of longer duration (2-3 minutes) that rely primarily on oxidative metabolisms, such as swimming and long-distance running, are classified as aerobic activities. Many sports activities require a combination of anaerobic and aerobic metabolism. In stop and go sports, about 60% -70% of energy comes from ATP storage from phosphocreatine (PCr) and anaerobic glycolysis, the remaining 30% from oxidative processes(⁶).
Phosphocreatine (PCr) is a high-energy compound that has a high-energy phosphate bond that can be hydrolyzed into energy and can resist ATP. In physical activity or strenuous exercise such as sprinting, PCr in the skeletal muscles makes a big contribution for the first 10 seconds. The storage of PCr will quickly run out but, in the first few seconds of exercise, PCr provides a significant buffer before other aspects of metabolism are activated(7).

During exercise, physical activity, or exercise, PCr depletion increases, and early intracellular acidosis has occurred. These changes contribute to a decrease in training capacity related to exercise duration(8).

**Material and Method**

This study used a literature review method. The article search strategy has carried out by using international or national journal articles that were searched through Google Scholar and PubMed. Through the keywords searched were exercise duration, exercise intensity, phosphocreatine, and PCr, from Google Scholar website founded 31 articles of search results and on the PubMed website as many as 13 articles. Then, screening has carried out by selecting articles that were relevant to the topic, namely the effect of exercise duration on phosphocreatine (PCr) levels. The articles selected were articles with inclusion criteria using Experiment research methods. The exclusion criteria in this literature review were articles that were not related to the topic of the effect of exercise duration and intensity on phosphocreatine levels.

![Consort diagram of research articles Effect of Exercise Duration and Intensity on Phosphocreatine Levels (PCr).](image)

**Results and Discussion**

Various types of sports can be an option to maintain body fitness. However, it is considerable to note in planning exercise activities that at least four criteria are met, namely the frequency of exercise, intensity/weight of exercise, type of sports activity, and length of time exercising(4). In sports, various kinds of metabolism will produce different types of waste products, one of which is creatinine.
Creatinine is a chemical waste molecule that results from muscle metabolism. Creatinine has produced from creatine, a molecule that is essential for energy production in muscles. About 2% of keratin in the body is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. Creatinine levels are determined by the amount of muscle mass (protein catabolism rate), in addition to how our body’s metabolic activity, for example, increases when we are sick (heat/infection). Creatinine is produced during skeletal muscle contraction through the breakdown of creatinine phosphate. Muscles use phosphocreatine during the first few seconds of intense muscle contraction, such as during weight lifting or sprinting. Unlike aerobic contraction, which utilizes oxygen to produce energy, phosphocreatine triggers energy without oxygen or is anaerobic.

Bogdanis et al. stated that aerobic metabolism provides a significant part (~ 49%) of energy during the second sprint, whereas PCr availability is important for high power output during the initial 10 s. This is supported by the statement of Hall and Trojan that creatine monohydrate can improve muscle performance in a short duration, and high-intensity resistance training will rely on the transport of phosphocreatine to become adenosine triphosphate so that an increase in the level of total creatine in cells will allow for the faster synthesis of phosphocreatine. Increasing the creatine level in the body can delay fatigue because creatine can be re-synthesized and sent back to the site of ATP use more quickly. Fatigue during short-term high-intensity exercise is related to the availability of PCr because PCr can regenerate ATP at very high rates, and its concentration in muscle is limited.

The energy at the start of a workout or exercise that uses very high-intensity muscle work (85% -100% of maximum capacity) and has a short duration (up to 10 seconds) has determined from the small amounts of ATP and PCr stored in muscle cells. The total energy available in the stored ATP-PCr is sufficient for short duration exercises, such as lifting weights, high jumps, or 10-second sprints.

The amount of energy generated from the PCr has limited because of the intramuscular pathway. The high anaerobic demands on the muscles can decrease muscle PCr concentration. At the same time, in a short period of maximum exercise, the anaerobic utilization of muscle PCr and glycogen will trigger muscle contraction. Short-term contractions are associated with metabolic changes in the muscle so as decreased muscle phosphocreatine.

According to Haseler, Hogan, and Richardson, in skeletal muscle, PCr recovery from submaximal exercise is a measure of muscle-oxidative capacity. PCr recovery was significantly altered by FIO2 and after submaximal exercise, in normoxic conditions, PCr recovery was limited by O2 availability. Also, The PCr recovery time constant is prolonged in patients with the symptomatic peripheral arterial disease (PAD), whether differences in PCr recovery time result entirely from changes in tissue blood flow, alterations in skeletal muscle at a cellular level, or a combination of both deserves further investigation.

In patients with PAD symptoms, the PCr recovery time constant is longer than that of ordinary people, so it is recommended for PAD sufferers to do sports with a long duration of intensity. Also, there is the notion that phosphate regulation and muscle O2 utilization is fully mature in peri-pubertal children, which may be due to the comparable capacity for mitochondrial oxidative phosphorylation in child and adult muscles.

The relatively higher rate of oxidative ATP build-up in children’s muscles to mask the ATP demand from high-intensity intermittent exercise compared to adults, allows children to start each exercise interval with a much higher concentration of PCr and lead to more muscle acidification. Low overall.

**Conclusion and Acknowledgement**

**Conclusion**

From the review above, it can conclude that PCr has a significant relationship with exercise duration and intensity. PCr is the largest energy contributor in the first 10 seconds of exercise in a heavy-intensity exercise where phosphocreatine triggers energy without oxygen or anaerobic, so with an increase in creatine phosphate capacity, very high-intensity exercise performance can be improved. PCr concentration is not only influenced by the duration and intensity of exercise, but also by
Acknowledgment: We thank all the panelists involved in this study. The researchers also extend the gratitude to Direktorat Riset dan Pengabdian Masyarakat, Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset dan Teknologi/ Badan Riset dan Inovasi Nasional for funding this study.

Conflict of Interest: No conflict of interest real or perceived.

Source of Funding: This article was funded by Direktorat Riset dan Pengabdian Masyarakat, Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset dan Teknologi/ Badan Riset dan Inovasi Nasional through Program Disertasi Doktor (PDD) grant 2020.

Ethical Clearance: The ethical clearance was obtained from The Committee of Ethical Approval in the Faculty of Nursing Universitas Airlangga No. 1974-KEPK.

References
Factors that Contribute to the QTc Interval Prolongation in DR-TB Patients on STR Regimen

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Abstract

Introduction: QTc interval prolongation is one of the adverse drug reaction of several drugs used in DR-TB patients treated with STR regimen. Drug-induced QTc prolongation can predispose patient to develop life-threatening arrhythmia, increasing hospital length of stay and mortality. This study aims to determine factors that contribute to QTc prolongation in DR-TB patients on STR regimen.

Methods. This was an observational retrospective study using medical records of DR-TB patients who received STR regimen from August 2017 to March 2019 in tertiary hospital DR Soetomo, Surabaya, Indonesia. QTc interval was calculated by Fredericia formula. The influence of risk factors (age, body weight (BW), body mass index (BMI), gender, comorbid, potassium, sodium and QTc baseline) with QTc prolongation was analyzed using multiple regression. The relationship between Moxifloxacin dosage and ΔQTc was analyzed using Chi-Square test.

Results Out of the 113 DR-TB patients who received the STR therapy regimen, 98 patients were eligible for this study. They consist of 62 (%) male; 36 (%) female. Thirty-five (35,7%) of them had Diabetes Mellitus as a comorbid disease. The mean age of the patients was 44±11 years, with the mean of BMI was 20.20±3.73. Potassium and Sodium levels at the baseline were 4.192 ± 0.58 and 138.05 ± 4.562 respectively. The QTc baseline before receiving STR regimen was 431.9±30,617ms. Patients received a dose of moxifloxacin 400 mg (5.1%), 600 mg (59,2%), and 800 mg (35,7%) according to body weight. There were no correlation between age, BW, gender, comorbid, potassium, sodium and QTc baseline with ΔQTc. There were correlation between potassium (p=0,001), BMI (p=0,006) and QTc baseline (p <0,001) with ΔQTc.

Conclusion QTc baseline and potassium level are factors that contribute to the prolongation of the QTc interval.

Keywords: QTc interval prolongation, STR regimen, Drug Resistance Tuberculosis (DR-TB)
with stagnant treatment success rates of roughly 54% and 30%, respectively. Despite adverse events associated with several DR-TB drugs, newly developed drugs and shorter regimens are bringing hope (1). However, it leads to a possibility that some factors may contribute to QTc prolongation in DR-TB patients on Shorter-Term Regimen (STR) treatment. A QTc >500 ms is considered a risk factor for ventricular arrhythmias, such as torsades de pointes (TdP), increasing hospital length of stay and mortality (2, 3). Overall, 10-20% of patients with drug-induced QTc prolongation have genetic predisposition, and >70% have at least two other risk factors (4). This assumes clinical importance in the presence of QT prolongation risk factors.

Drug-induced QTc prolongation is characterized by acquired QT interval prolongation and may be followed by potentially fatal proarhythmias known as torsades de pointes, which can result in sudden cardiac death (5, 6). Drug-induced QTc prolongation is often dose-related (7). Depending on their dosages, certain drugs may prolong the duration of ventricular action potential and the QT interval by means of different ionic mechanisms. Most drugs that prolong the QTc interval act by blocking hERG-encoded potassium channels, although some drugs modify sodium channels (8). Hypokalemia might be one of the most important risk factors for QT prolongation since some studies revealed that hypokalemia were associated with lengthening of the QT interval (9-11). As a result of Hypokalemia, high level of sodium (Hypernatremia) may cause the same effect (12). In the previous study, baseline of QTc was an important predictive marker of QTc prolongation in patients with Diabetes Mellitus during Severe Hypoglycemia (13). Age is other factor that may cause QTc prolongation in DR-TB patients on STR regimen. In a healthy subjects, age significantly correlated with QT and QTc interval (14). Prolonged QTc is more prevalent in older age (15). QT Interval prolongation is common in obesity and shortens with weight loss (16, 17). In line with body weight, those with higher BMI have a significantly longer QTc (18, 19). In many studies, patients with Diabetes Mellitus comorbid had QTc prolongation as compared to those without it (20, 21). Last but not least, gender is a factor that can also be one of QT prolongation risk factors. The relationship between gender and QT interval using administration of cardiovascular drugs showed that women are more prone than men to develop TdP (22).

Better knowledge of the QTc prolonging in relation to risk factors is needed to improve decision-making. Even though there are data on some factors that may contribute to QTc prolongation, but very little information about the risk factors in Drug-Resistant Tuberculosis (DR-TB) patients, especially during Short-Term Regimen (STR). In this study, we analyzed the effect of the risk factors on the length of the QTc interval in a hospital population. This study aims to determine factors that contribute to QTc interval prolongation in DR-TB patients on STR regimen. Besides the use of TB drug that are known to prolong the QTc interval, we analyzed the effect of the additional risk factors on the QTc interval, such as age, gender, electrolyte disturbances, comorbid (Diabetes Mellitus), body weight (BW), Body Mass Index (BMI), drug dosage, and the baseline of QTc.

**Methods**

**Study population and design**

We performed a retrospective observational study. The study population was recruited and analyzed from the medical records of Drug-Resistant Tuberculosis (DR-TB) patients who received Short-Term Regimen treatment, diagnosed from August 2017 to March 2019, was undertaken at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The diagnosis of pulmonary TB in hospitals and TB clinics is made on the basis of clinical examination, chest radiography, rapid test molecular, and sputum smear microscopy and/or sputum culture (23).

**Data collection**

Medical records of Drug-Resistant Tuberculosis (DR-TB) patients who received Short-Term Regimen treatment, diagnosed from August 2017 to March 2019 at tertiary hospital DR Soetomo, Surabaya, Indonesia were used as the data source. We collected and divided data of following risk factors on the length of QTc interval into groups: gender (female and male), age, comorbid, Body Mass Index (BMI), potassium, natrium, baseline QTc. Patient with missing serial ECG, incomplete medical record are excluded from this study

**QTc interval measurement and interpretation**

ECGs were recorded at baseline or pre-treatment, two weeks post-treatment. increase of 10mm/mV and
paper speed of 25 mm/s. Standard supine 10 s, 12-lead resting ECG was recorded with a digital ECG Biolight E30 channel with interpretation. The ECG parameters/ intervals that were assessed at each visit were heart rate (beats/minute), PR, QRS, QT, and QTc intervals (ms). All QT values were double checked by visual examination.

QT interval was measured from the beginning of the QRS complex to the end of the T-wave in the derivation where the QT interval was the most visible. QTc interval (baseline and follow up QTc) was calculated by Frederica formula (QTcFri = QT/RR\(^{1/3}\)) used to count QT correction \((24)\). QTc prolongation classified according to the Common Terminology Criteria for Adverse Events (CTCAE) guidelines version 4.03 (grade 0, QTc <450; grade 1, QTc 450-479 ms; grade 2, QTc 480-499 ms; grade 3, QTc > 500 ms; grade 4, QTc >500 ms with life-threatening signs or symptoms \((25)\).

Single delta QTc interval denoted as ∆QTc. It estimates the differences in QTc of two ECG signal. In this study we measured QTc pre dose or baseline QTc and QTc Postdose. The formula of ∆QTc is QTc\(_{\text{day0}}\) minus QTc\(_{\text{day14}}\). Based on ICH E14 Guideline divided ∆QTc as QTc interval increases from baseline >30 msc and >60 msc \((26)\).

**Study Drug**

STR is 9-month regimen consists of kanamycin, ethionamide, moxifloxacin, clofazimine, ethambutol, and high dose isoniazid \((27)\). Moxifloxacin of 400 mg/tablet (Avelox®, Bayer HealthCare) was used. Dosing moxifloxacin based of body weight. The dosing material were stored at 25° in a dry location.

**Data Analysis**

The statistical package SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used to analyze data. The influence of risk factors (age, body weight (BW), Body Mass Index (BMI), gender, comorbid, potassium, sodium and QTc baseline) with ∆QTc prolongation was analyzed using multiple regression. The relationship between Moxifloxacin dosage and ∆QTc was analyzed using Chi-Square test. Slope test between ∆QTc and baseline QTc using scattered plot.

### Results

#### Study Demographics and Disposition

The study population was composed of 62 males and 36 females. The mean age of subject was 44±11 years old (males 44±12; females 44±10), and their body mass index was 20.2±3.7 kg/m\(^2\) (males 20±3.2; female 20±4.4). Several factors could modify the risk of ∆QTc prolongation such as gender, comorbid, BMI, potassium, sodium, and baseline QTc. Based on multiple regression model, there is no significant correlation between age, gender, comorbid, and sodium level \((p<0.05)\). Interestingly subject with underweight BMI statistically significant with ∆QTc prolongation \((p=0.006; \ CI 95\% -0.07 – -0.13)\) rather than overweight patients. It probably due to distribution of drug in fat tissue.

Subject with low potassium level 3.8±0.7 will increase ΔQTc prolongation \((p=0.001; \ CI 95\% -0.53 – -0.15)\). Low baseline QTc also \((p<0.001; \ CI 95\% -0.015 \ -0.008)\) statistically significant with ΔQTc prolongation (table 1).

#### Categorical analysis of QTcF

The result of categorical analysis of the QTcF and ΔQTcF are summarized in table 2. QTcF of >500 ms observed in two subject with 800 mg of moxifloxacin. QTcF of >480 and <500 observed in three subject with 600 mg of moxifloxacin. ΔQTcF of >60 ms observed in one patient with 400 mg moxifloxacin and nine with 600 mg. ΔQTcF was >30 and ≤60 ms in 21 subject receiving moxifloxacin with one patient in 400 mg, 12 subject with 600 mg and eight subject in 800 mg dosage.

There is no substantial variation prolong ΔQTc between moxifloxacin 600 mg and 800 mg, but the incidence of prolong ΔQTc is higher in 600 mg moxifloxacin (nine patients ΔQTc >60 msc). Based on statistical analysis, there are no significance between baseline QTcF with moxifloxacin dosage \((p=0.283)\) and ΔQTcF \((p=0.176)\).

The linear relationship between baseline QTc prolongation and ΔQTcF with 95% CI are shown in figure 1 which demonstrating negative slope for total subjects. Data showed low baseline QTc would increased ΔQTcF.
### Table 1. Correlation between risk factor with ∆QTcF

<table>
<thead>
<tr>
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<th>∆QTcF</th>
<th>p</th>
<th>CI 95%</th>
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<tr>
<td></td>
<td>≤30 (n=67)</td>
<td>&gt;30 (n=21)</td>
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<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>Man</td>
<td>42</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Woman</td>
<td>25</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Age</td>
<td>44 ±11</td>
<td>44 ±10</td>
<td>42±16</td>
</tr>
<tr>
<td>Comorbid</td>
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<td></td>
</tr>
<tr>
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<td>26</td>
<td>8</td>
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</tr>
<tr>
<td>No</td>
<td>41</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>BMI</td>
<td>20.8±4</td>
<td>19.3±3</td>
<td>17.7±1.5</td>
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<tr>
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<td>4.3±0.5</td>
<td>3.9±0.5</td>
<td>3.8±0.7</td>
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<tr>
<td>Natrium</td>
<td>138±4</td>
<td>137±5</td>
<td>138±5</td>
</tr>
<tr>
<td>Baseline QTcF</td>
<td>442±23</td>
<td>418±22</td>
<td>392±45</td>
</tr>
<tr>
<td>QTc week 1 after drug</td>
<td>437±22</td>
<td>463±20</td>
<td>492±53</td>
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</table>

### Table 2. Correlation between moxifloxacin dosage with categorical baseline QTc and ∆QTcF

<table>
<thead>
<tr>
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<th>Moxifloxacin dosage</th>
<th>p</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400 (n=5)</td>
<td>600 (n=58)</td>
<td>800 (n=35)</td>
</tr>
<tr>
<td>Baseline QTcF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤450</td>
<td>5</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>&gt;450</td>
<td>0</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>&gt;480</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>&gt;500</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>∆QTcF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤30</td>
<td>3</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>&gt;30</td>
<td>1</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>&gt;60</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>
Discussions

QT interval on the electrocardiogram (ECG) represents the action potentials in ventricular myocytes. Mechanism of QT interval prolongation is result from an increase in inward current (e.g., through sodium or calcium channels) or a decrease in outward current (e.g., through potassium channels) into action potential prolongation (28). From this study, we found several risk factor might contribute ∆QTc prolongation. Low BMI, hypokalemia, and baseline QTc can cause ∆QTc prolongation.

Low body mass index affected QTc prolongation possibly by decreased left ventricular mass and cardiac chamber dimension (29). Abnormal ion transport may also occur in malnourished cells independent of absolute serum electrolyte concentrations (30). Mischisita et al was finding that the QTc interval was significantly longer in the low BMI groups compared to the moderate BMI group in both genders (31). It has been well known that a prolonged QTc interval is reflected in the dysfunction of the cardiac autonomic nervous system, while the cardiac autonomic nervous system is influenced by eating disorders.

Hypokalemia induced changes in ECG are probably qualitatively similar with action potential duration (APD) (32). Low extracellular potassium enhanced inactivation and reduces IKr or increase competitive block by sodium. As a result, hypokalemia prolongs the QT interval (28).

Prolongation of QT interval may be noted when there is a delay in myocardial repolarization secondary to ionic currents from electrolyte abnormalities. Phase 3 of myocardial repolarization is predominantly mediated through delayed outward rectifier potassium currents (IKr and IKs) which are in turn dependent on extracellular potassium concentration. In case of hypokalemia, there
is decreased expression of these channels resulting in prolongation of repolarization (33).

The QTc interval may confirm the hypothesis that a low potassium leads to the occurrence of future cardiac sudden death and the incidence of CVD. Based on our results, we consider that it is necessary to perform dietary counseling, especially focusing potassium intake, depending on the body mass.

This study didn’t had significance relationship between dosage of moxifloxacin with ∆QTc. We suggest that every dosage moxifloxacin can occur QTc prolongation. Previous reports have suggested that patients developing drug-induced long QT syndrome with one drug are more likely to develop drug-induced long QT syndrome with exposure to other drugs (34). Other drug might contribute to QTc prolongation in shorter regimen is clofazimin (35).

**Conclusions**

Based on the results of this study, low body weight, hypokalemia, QTc baseline and QTc after 1-week after drug admission had significance effect for ∆QTc prolongation. We suggest frequent ECG monitoring to individual on STR therapy especially patient with risk factor that contribute ∆QTc prolongation.

**Funding:** None.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethical Approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

**Acknowledgement:** We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

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Discordance between Genexpert, Line Probe Assay and Drug Susceptibility Test in Assessing Drug-Resistant Tuberculosis

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Abstract

Background: Rapid molecular diagnostics have potentially revolutionized early detection of drug-resistant tuberculosis (DR-TB) in Indonesia. However, there is discordance between conventional culture using drug susceptibility test (DST) and rapid diagnostic tools using GeneXpert and line probe assay (LPA). This discordance result can cause confusion to clinician in determining diagnosis of DR-TB. Objective: This study aimed to identify discordance between GeneXpert, LPA, and DST. Methods: A retrospective study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Data were collected based on medical record between third to fourth quarter of 2018. Rifampicin-resistant-tuberculosis (RR-TB) proven by GeneXpert, were further analyzed with second-line LPA and DST. Discordance result between it was analyzed using McNemar. Results: Among 81 patients diagnosed with DR-TB, 59 patients RR-TB were eligible in this study. There were 13 (22.0%) DST rifampicin result showed sensitive but resistant to GeneXpert. Among 53 samples from LPA, there were 3 (5.7%) result showed LPA fluoroquinolone resistant but sensitive to DST ofloxacin; 10 (18.9%) result has LPA fluoroquinolone sensitive but resistant to DST ofloxacin; 2 (3.8%) has LPA second-line injectable drug (LPA SLID) resistant but sensitive to DST kanamycin; 5(9.4%) has LPA SLID sensitive but resistant to DST kanamycin. The McNemar analysis showed discordance between GeneXpert and DST rifampicin was 13 (22.0%; p=0.046); LPA fluoroquinolone and DST ofloxacin was 13 (24.6%; p=0.042); LPA SLID and DST kanamycin was 7 (13.2%; p=0.183). No variable that can be used to analyze discordance result between GeneXpert and LPA. Conclusion: There is significant discordance between GeneXpert and DST; LPA fluoroquinolone and DST ofloxacin, while neither LPA SLID nor DST kanamycin shows no significant discordance.

Keywords: Rapid test, conventional culture, drug susceptibility test, line probe assay, drug-resistant tuberculosis

Introduction

Worldwide, tuberculosis (TB) is one of the top 10 causes of death and the leading cause of curable single infectious agent1. It is an infectious disease caused by the bacillus Mycobacterium tuberculosis (MTB)2. Now we stand at Sustainable Development Goals (SDGs) for 20303. TB is the only disease ever declared a global emergency by the World Health Organization (WHO)2. Integral to this transition, the world community is launching accelerated fight against TB3.

Globally in 2017, around 10.0 million people (range 9.0 – 11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women, and 1.0 million children. Drug resistant-tuberculosis (DR-TB) continues to be a public health crisis1. The emergence of DR-TB is further complicating the situation and is threatening
to jeopardize all the prior gains by global TB control programs in recent years. The best estimate is that 558,000 people (range 483,000 – 639,000) developed TB that was rifampicin resistant-tuberculosis (RR-TB), the most effective first line drug, and of these, 82% had multidrug resistant-tuberculosis (MDR-TB). Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/RR-TB. Among cases MDR-TB in 2017, 8.5% were reported to have extensively drug resistant-TB (XDR-TB).

DR-TB surveillance data show that an estimated 160,684 cases of MDR/RR-TB were detected and notified. Of these, a total of 139,114 people (87%) were enrolled on treatment with a second line-regimen. In spite of increased testing from 129,689 cases in 2016, but still only 25% of the estimated 558,000 people who developed MDR/RR-TB.

TB is an age old disease, but even today the diagnosis of TB remains elusive. Urgent action is required to improve the quality of diagnosis for people with DR-TB. Indonesia accounted for countries for 11% from 80% of the 3.6 million global gaps in the detection and treatment of TB cases. In 2017, Indonesian national study found that although about 80% of new cases were detected, 41% of these cases were not reported. Gaps between the estimated number of new cases and the number actually reported due to underdiagnoses. Closing gaps in detection require much higher coverage of drug susceptibility testing among people diagnosed with TB, reducing underdiagnoses of TB.

Only one in every six estimated cases was being detected worldwide. Gap remains of the estimated MDR/RR-TB cases still undetected. For the diagnosis of TB, a large number of tests are available, each one having its advantages and disadvantages.

Culture based-methods remain the ‘‘gold standard’’ for TB diagnosis in developing countries as these techniques have been greatly improved and routinely used over the past decade. However, the time for bacteriological culture-based diagnosis of TB may require several weeks to months. To address such delay in TB diagnosis as well as to discretely upgrading the speed and quality of MTB diagnostic accuracy.

Worldwide emphasizing the need to be considered for the early detection of MTB which involves the detection of the mutation in specific genes imparting against resistance. Molecular methods have led to the development of rapid and reliable diagnostic and drug susceptibility testing. GeneXpert and Line Probe Assay (LPA) are two which approved standard molecular diagnostic methods that have been developed for the rapid detection of drug resistance by scanning the DNA for associated mutations. GeneXpert is an urgent necessity for tests that can quickly diagnose TB. Introduction of newer and rapid diagnostic tools have increased the detection of RR-TB cases. RR-TB is crucial for proper control of TB disease. In the present study comparative analysis of the conventional method and molecular method like LPA for diagnosis of MTB and detection of MDR TB is carried out.

However, there is a discordance between conventional culture with DST and rapid diagnostic tools with GeneXpert and LPA. This discordance result can cause confusion to the clinician in determining diagnosis DR-TB. Here, this study was observed the rapid molecular diagnostic validity of GeneXpert and LPA whether the results will or will not be in accordance with the results given by DST.

Methods

Study Design and Setting: A retrospective study conducted between third to fourth quarters of 2018 at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The subjects were 81 patients diagnosed with DR-TB. Only 53 patients were eligible for this study (figure 1).

Figure 1. Study flow diagram

This study reviewed based on medical records of diagnostic tools in DR-TB. DR-TB definition adapted from WHO guidelines. This study obtained data on demographics, gender, age, comorbidities, acid fast bacilli (AFB), GeneXpert, second line LPA, and DST. The inclusion criteria were men and women aged between 20 and 65 years. The exclusion data were that patient sample with incomplete GeneXpert, LPA, and DST data.

Procedure: Each sample data was initially examined with smear AFB sputum specimens. AFB data
were conducted from microbiology sputum examination result in the medical record. Positive AFB stains were quantified into 4 groups as scanty (1–9 AFB/100 fields), 1+ (1–9 AFB/10 fields), 2+ (1–9 AFB/fields), and 3+ (>9 AFB/fields).

Then sputum specimens were screened for DR-TB. Diagnostic tests for DR-TB disease include rapid molecular tests and culture based methods. The rapid test for diagnosis of TB currently recommended by WHO is the GeneXpert and LPA.

GeneXpert data were conducted from medical records. GeneXpert can provide results within 3 days, and was for diagnosis of pulmonary TB in adults. RR-TB proved by GeneXpert. GeneXpert RR-TB result was quantified into 4 groups as very low, low, medium, and high.

The further analyzed with second-line LPA. Second-line LPA data were collected from TB03. Second-line LPA sample comes from Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. Second-line LPA can provide results within 14 days. Second-line LPA result was targeting two drugs for its resistance, LPA fluoroquinolone (LPA FQ) and LPA second line injection drug (LPA SLID).

DST was performed on the culture-based methods to identify *Mycobacterium tuberculosis* complex (MTBC) strain. DST has carried to all the culture positive samples by Standard Proportion method. The drugs were used for DST, rifampicin, Isoniazid, ethambutol, streptomycin, kanamycin, amikacin and ofloxacin (Ofl), for DR-TB detection. DST form the current reference standard and can take up to 12 weeks to provide results2.

We compared DST as the gold standard with GeneXpert. We wanted to see concordance or discordance for detection of rifampicin resistance. We compared DST with second-line LPA. We wanted to see concordance or discordance for detection of fluoroquinolone and second line injection drug resistance.

Statistical Analysis: All of the patient’s data were collected on Microsoft Excel. Categorical variables were expressed as an absolute number. Statistical analysis was analyzed using IBM SPSS software 20.0 (IBM Corp., Armonk, NY, USA) for Windows. The cross-tabulation with McNemar formula was performed to analyze concordance or discordance between GeneXpert, second line LPA, and DST. The probability levels p < 0.05 were considered as statistically significant.

Results

The study has enrolled 81 patients diagnosed with DR-TB at study entry. Only 59 patients with RR-TB were eligible in this study.

Their demographic data are summarized in Table 1. In this research, baseline characteristic stratified by gender, approximately 69.5% of patient were men. The mean age of patients was 44.47 ± 11.27, range between the youngest ages was 22 years, while the oldest was 62 years. Around 72.9% of patients self-reported had common comorbidities. The most frequent patient comorbidities were smoking (54.2%) and diabetes mellitus (38.9%).

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>41 (69.5)</td>
</tr>
<tr>
<td>Women</td>
<td>18 (30.5)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≤ 60 years old</td>
<td>56 (94.9)</td>
</tr>
<tr>
<td>&gt; 60 years old</td>
<td>3 (5.1)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>43 (72.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23 (38.9)</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>6 (10.2)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>32 (54.2)</td>
</tr>
<tr>
<td></td>
<td>5 (8.5)</td>
</tr>
</tbody>
</table>

Sputum microscopy AFB showed 74.5% has a positive result. GeneXpert was stratified according to four categories very low, low, medium, and high rifampicin resistance. Most of sample 42.4% has a medium result. Second line LPA and DST were stratified according to two categories resistant and sensitive. 53 samples were analyzed for DR -TB by second-line LPA by targeting two drugs for its resistance, LPA fluoroquinolone (LPA FQ) and LPA second line injection drug (LPA SLID). It was found that 84.7% of samples were found to be sensitive by second-line LPA. Also, only 5.1% of the
samples were found to be resistant by second-line LPA. DST component was isoniazid rifampicin, ethambutol, streptomycin, kanamycin, amoxicillin, and ofloxacin. Resistant DST rifampicin was 78.0%, resistant DST kanamycin was 8.5%, and resistant DST Ofl was 18.6%. The other laboratory result is summarized in Table 2.

### Table 2. DR-TB based on laboratory modality (GeneXpert, LPA, and DST)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Frequency (%)</th>
<th>Resistant (%)</th>
<th>Sensitive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on AFB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10 (16.9)</td>
<td>46 (78.0)</td>
<td>14 (23.7)</td>
</tr>
<tr>
<td>Scanty</td>
<td>5 (8.5)</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td>1+</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td>2+</td>
<td>18 (30.5)</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td>3+</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td>Based on GeneXpert</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very low</td>
<td>6 (10.2)</td>
<td>0 (0.0)</td>
<td>6 (10.2)</td>
</tr>
<tr>
<td>Low</td>
<td>15 (25.4)</td>
<td>15 (25.4)</td>
<td>15 (25.4)</td>
</tr>
<tr>
<td>Medium</td>
<td>25 (42.4)</td>
<td>25 (42.4)</td>
<td>25 (42.4)</td>
</tr>
<tr>
<td>High</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td>Based on LPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTB positive</td>
<td>53 (89.8)</td>
<td>3 (5.1)</td>
<td>50 (84.7)</td>
</tr>
<tr>
<td>LPA FQ</td>
<td></td>
<td>3 (5.1)</td>
<td>50 (84.7)</td>
</tr>
<tr>
<td>LPA SLID</td>
<td></td>
<td>3 (5.1)</td>
<td>50 (84.7)</td>
</tr>
<tr>
<td>Based on DST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>38 (64.4)</td>
<td>15 (25.4)</td>
<td>15 (25.4)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>46 (78.0)</td>
<td>13 (22.0)</td>
<td>13 (22.0)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>14 (23.7)</td>
<td>39 (66.1)</td>
<td>39 (66.1)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>13 (22.0)</td>
<td>40 (78.0)</td>
<td>40 (78.0)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>5 (8.5)</td>
<td>54 (91.5)</td>
<td>54 (91.5)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>6 (10.2)</td>
<td>53 (89.8)</td>
<td>53 (89.8)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>11 (18.6)</td>
<td>48 (81.4)</td>
<td>48 (81.4)</td>
</tr>
</tbody>
</table>

There were 13 (22.0%) DST result showed sensitive rifampicin but resistant form GeneXpert. Among 53 samples from second line LPA, there were 2 (5.7%) result showed resistant to fluoroquinolone (FQ) but sensitive to ofloxacin from the DST; 10 (18.9%) result has FQ sensitive but resistant to ofloxacin from DST; 2 (3.8%) has second line injectable drug (SLID) resistant but sensitive to kanamycin from DST; 5 (9.4%) has sensitive SLID but showing kanamycin resistant from DST.

### Table 3. Concordance and discordance between GeneXpert on DST and second-line LPA on DST

<table>
<thead>
<tr>
<th>Diagnosis DR-TB</th>
<th>Resistant (%)</th>
<th>Sensitive (%)</th>
<th>Concordance (%)</th>
<th>Discordance (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneXpert RR</td>
<td>46 (78.0)</td>
<td>13 (22.0)</td>
<td>46 (78.0)</td>
<td>13 (22.0)</td>
<td>0.046</td>
</tr>
<tr>
<td>LPA FQ</td>
<td>0 (0.0)</td>
<td>3 (5.7)</td>
<td>40 (75.4)</td>
<td>13 (24.6)</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>10 (18.9)</td>
<td>40 (75.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPA SLID</td>
<td>0 (0.0)</td>
<td>2 (3.8)</td>
<td>46 (86.8)</td>
<td>7 (13.2)</td>
<td>0.183</td>
</tr>
<tr>
<td></td>
<td>5 (9.4)</td>
<td>46 (86.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The analysis showed discordance between GeneXpert and DST rifampicin was 13 (13.2%) samples with \((p=0.046)\). Discordance between LPA fluoroquinolone and DST ofloxacin was 13 (24.6%) samples with \((p=0.042)\); LPA SLID and DST kanamycin was 7 (13.2%) samples with \((p=0.183)\). There is no variable that can be used to analyze the discordance result between GeneXpert and second line LPA (table3).

**Discussions**

In the recent years, major importance has been given on rapid diagnosis and quick initiation of accurate treatment for DR-TB\(^8\). Resistance to anti-TB drugs can occur when these drugs are misused or mismanaged\(^{10}\). Late diagnosed DR-TB leading to diagnostic delay with associated exacerbation of transmission, amplification of resistance, and increased mortality\(^{11}\). Precise and early diagnosis of DR-TB is extremely beneficial as it interrupts further transmission of the disease and avoids addition of life-saving drugs and consequently increases of drug resistance. It also avoids unnecessary cost of administration and occurrence of serious side effects of second line anti-TB drugs in case one is dealing with drug sensitive MTB strains\(^2\).

The best method of diagnosing an infectious disease is to demonstrate the causative organism in representative samples of tissue or fluid by either staining and or by culture. Sputum microscopy by the AFB staining is accepted worldwide as the first line test as it is simple, convenient, rapid, inexpensive and can be done in field condition. However, it is less sensitive as it requires bacterial load at least 10\(^4\) bacilli per ml of sputum to be positive and it may be falsely positive in many conditions including environmental mycobacterial infection\(^6\). AFB cannot distinguish between dead and live bacteria and is unable to identify different species of MTB\(^2\). Culture is the reference golden standard for TB diagnosis but they are time consuming. Culture and drug-susceptibility testing (DST) using solid media can take up to 8–12 weeks for results and faster liquid-based culture techniques still take at least 4–6 weeks\(^5\). DST based on the estimation of growth or no growth of an MTB strain in the presence of a single critical concentration of one drug. The critical concentration of an anti-tuberculosis drug represents the lowest concentration of the drug in the medium that indicates clinically relevant resistance if growth is observed. Susceptible wild-type strains are inhibited by this concentration. Resistance is defined if over 1% of the bacterial population of a strain is able to grow\(^2\).

The development of rapid molecular diagnostic tests for the identification of MTB and drug resistance has consequently become a research and implementation priority\(^5\). Rapid and accurate diagnosis of pulmonary TB remains a great challenge. There is an urgent necessity for tests that can quickly diagnose TB. Hence the most promising approach was to demonstrate remnants of the TB bacilli in representative samples. Detecting even small amounts of bacterial DNA was feasible due to the development of various molecular diagnostic tests for TB\(^6\).

Molecular techniques have revolutionized the diagnosis of pulmonary tuberculosis (PTB), as well as DR-TB. Rifampicin resistance is considered as surrogate marker of DR-TB\(^6\). GeneXpert and LPA are recommended for diagnostic testing for the presence of MTB and detection of mutations associated with rifampicin resistance\(^12\).

GeneXpert is a novel integrated diagnostic system for major change in the speed, simplicity and accuracy of not only diagnosis of TB but also drug resistance to rifampicin in TB, which is accepted as a surrogate for DR-TB. The rapidity and robustness of diagnosis in-turn breaks the chain of transmission in addition to early institution of treatment and improved chances for cure\(^13\). GeneXpert can simultaneously identify MTB and rifampicin resistance within two hours. The GeneXpert has been approved by the WHO in 2013. It adopted a GRADE system approach to arrive at recommendations on the diagnostic value of the assay in PTB patients on therapy for less than seven days. This test has the potential to dramatically reduce the time to diagnosis and the time to initiation of effective therapy\(^6,13\).

GeneXpert advantage is simple and automated to perform with minimal training, is not prone to cross-contamination, and requires minimal biosafety facilities\(^4,6\). GeneXpert requires a reliable power supply and operating temperatures below 30\(^\circ\)C. Sputum should be of good quality, and it should be concentrated by usual laboratory methods\(^6\). The specificity and sensitivity of GeneXpert for detection of rifampicin resistance are
more than 98% and 99%, respectively\textsuperscript{4,14}. GeneXpert is designed to identify rifampicin resistance\textsuperscript{6}. Rifampicin is a widely used first line anti TB drug that works by inhibiting the mycobacterial ribonucleic acid (RNA) synthesis\textsuperscript{4}. The vast majority (around 95 to 98%) of rifampicin resistance associated mutations using DNA probes in an 81-bp region (codons 507 to 553) of the RNA polymerase β subunit (rpo-β) gene known as the rifampicin resistance determining region (RRDR). Rifampicin resistance detection in the GeneXpert is based on hybridization or the absence of five molecular beacon probes complementary to the wild type sequence of rpo-β gene\textsuperscript{14}. Most of the RIF resistance mutations are of the first kind hence are easily detected\textsuperscript{15}.

In our study, there is discordance between GeneXpert and DST Rif, but the percentage is low. The accuracy for identification of rifampicin resistance was 98%. However, a study done in Swaziland demonstrated that the assay may not be able to detect wild type mutations for rifampicin resistance outside the rpo-β I491F domain\textsuperscript{6,4}. GeneXpert detected four samples discordant with culture DST. On sequencing, two showed mutations [517,519 [dual mutation] and 533CCG], while two others had no mutation in the RRDR. The mutations at probe ends might be missed\textsuperscript{13}.

Another study revealed mixed MTB infections have been suggested to be responsible for false negative and positive results for rifampicin resistance. Several studies have reports of mixed infections, its interference with drug resistance detection. For the same reason, GeneXpert cannot be used for assessing the emergence of rifampicin resistance during treatment. Hetero-resistance MTB populations is often suggested to be responsible for discordant DST results\textsuperscript{13}.

LPA are rapid molecular diagnostics that can detect MTB and drug resistance. Although LPAs are more technically complex and take longer to perform than the GeneXpert. First line LPA detect drug resistance by identifying mutations in the rpo-β, katG, and inhA genes. By targeting mutations in the 81-base pair “core region” of the rpo-β gene, more than 95% of all Rifampicin resistant strains can be detected. Although mutations in katG and inhA account for approximately 80-90% of INH resistant strains\textsuperscript{5}.

LPA targeting resistance to second line anti TB drugs are under evaluation. Second line LPA detect fluoroquinolone (moxifloxacin) and SLID (aminoglycosides, kanamycin, and capreomycin) resistance by identifying mutations in gyrA, and rrs. Sequencing of the gyrA, and rrs genes was performed on a representative sample of isolates with discrepant second line LPA and DST results. These assays detect mutations in the gyrA gene as fluoroquinolone resistance and detected mutation in rrs gene as kanamycin, Amk, and Cm resistance\textsuperscript{11}.

In the present study we have performed a comparison of the conventional method of DST and a newer molecular method that is LPA for the detection of DR-TB from the sputum samples\textsuperscript{2}. In Indonesia we have only check second-line LPA. The second-line LPA detects resistance to fluoroquinolones (LPA-FQ) and second-line injectable drugs (LPA-SLID), and it may be used as an initial test for second-line drug resistance\textsuperscript{6}. The results of LPA were then compared to the DST, which is still a gold standard\textsuperscript{2}. A positive result is reliable for detection of drug-resistant TB but a negative result may not always rule out the presence of drug-resistant TB, and that should be confirmed by conventional culture and drug sensitivity test (DST)\textsuperscript{6}.

LPA is capable of indicating hetero-resistance but the limit of detection of LPA is at least 5x10\textsuperscript{3} bacilli per ml of sample, hence the bacillary load would need to be high enough for detection using reverse hybridization by LPA. The LPA also may not detect all mutations at position 533; the probes are so designed that the mutation does not always affect the loss of binding of probes\textsuperscript{13}.

Another study discordance between LPA and DST may be due to DNA extraction protocol of LPA. In LPA, DNA extraction was done directly from the sputum samples; hence even the DNA of dead bacilli may have contributed. Higher percentage of TB positive in LPA may be attributed to the limitation of the technique that cannot differentiate between live and dead bacilli\textsuperscript{2}.

Depending on the specific region interrogated by SL-FL and SL-LPA, one or more follow-up diagnostic actions are either recommended or suggested as optional to better guide the choice of the treatment regimen. The decision to perform the optional follow-up diagnostic actions should be guided by considerations on the individual patient’s risk for resistance and by the
prevalence of resistance in the specific geographical setting, as these factors affect the positive predictive value of the test\textsuperscript{16}. Genotypic testing is much faster than phenotypic methods, as these are not growth based tests. Drug sensitivity test results by solid Lowenstein Jansen media has a turnaround time of up to 84 days, liquid culture up to 42 days, LPA up to 72 hours and GeneXpert by 2 hours\textsuperscript{6}.

However, it should be remembered that a positive result suggest but a negative result do not exclude TB as well as DR-TB. At present GeneXpert and LPA has not totally replaced the traditional smear and culture for TB\textsuperscript{6}. Molecular tests are not recommended for treatment monitoring. DST may be used during treatment to assess for any acquisition of additional resistance or reinfection. Given that decisions on the treatment of patients depend to an important degree on the bacteriological findings\textsuperscript{12}.

**Conclusions**

Bacteriological examinations in patients with DR-TB include sputum smear microscopy, culture and DST as well as rapid test such as GeneXpert and LPA. Genotypic assays though offer rapidity and most often a good sensitivity when the probes designed can cover all possible mutations responsible for resistance but could give false positive results due to detection of mutations not responsible for resistance. There is significant discordance between GeneXpert and DST; LPA fluoroquinolone and DST ofloxacin, while neither LPA SLID nor DST kanamycin shows no significant discordance.

**Acknowledgements**

We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

**Conflict of Interest**: The authors declare that they have no conflict of interest.

**Author's Contribution**

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

**Funding**: None

**Data Availability**: The dataset used and/or analyzed during the current study are available from corresponding author on reasonable request.

**Ethics Statement**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1491 / KEPK / IX / 2019).

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Complex Post Traumatic Stress Disorder (CPTSD) for University Students

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Abstract

Complex posttraumatic stress disorder is a diagnostic construct which tries to capture complex trauma reactions in face protracted and repeated psychological trauma. These reactions which compromise specific symptom constellation may be missed if there is focus on classical posttraumatic syndrome. Iraqi population endured four decades of instability which created atmosphere of protracted repeated trauma situation which necessitate examining complex trauma reactions. The aim was to create a tool for examining complex trauma reactions within Iraqi population and examining rates and nature of these reactions in a sample of Iraqi population. Random sample of medical students were examined for exposure to traumatic events and complex psychological trauma reactions. The tool used for examining complex trauma was made according to criteria of disorders of extreme stress not otherwise specified; validity and reliability of the tool was verified and rates of traumatic events and rates and nature of reactions were examined. Most of participants confirmed exposure to more than one traumatic event and 25% fulfilled criteria of complex trauma syndrome. Rates of complex trauma reactions are high in this study. Large population studies are needed to confirm this fact, which means extensive efforts are needed at clinical and social levels for proper help to be provided to traumatized individuals.

Keywords: CPTSD, PTSD, DESNOS

Introduction

Posttraumatic stress disorder PTSD was first introduced as psychiatric diagnosis in DSM III at 1980. Its introduction was based on available literature about psychological trauma of adults at circumstances of war and natural disasters and over later years research has confirmed PTSD as diagnostic category and supported its validity, reliability and applicability to clinical demands of trauma population. In the same time, such research continuously brought new looks at the diversity of psychological trauma reactions. The aims of this study are to build new questionnaire for complex psychological trauma reaction suitable for Iraqi population according to the six domains of DESNOS. Another aim is to detect the rates of complex trauma syndrome and its constituting symptoms in a sample of Iraqi medical students.

Literature Review

While the classical PTSD concept requires the triad of trauma re-experience, emotional numbing and over arousal, an increasing amount of literature found that such triad do not capture many other psychological reactions of trauma and reactions to trauma were found to be variable according to developmental age at exposure as well as to nature, frequency, duration and context of the trauma. Patients exposed trauma at early age or those exposed to frequent serious traumatic events of long duration or patients traumatized within the context of intimate relationship were found to show more complex reaction than the initial triad introduced with PTSD. It was found that co morbidity was common among patients with PTSD. Questions were
aroused whether this indicates that PTSD could lead to other conditions or there is need to review the diagnostic construct of PTSD. Soon then the concept of complex trauma reactions was described. DSM IV delineated a syndrome named disorders of extreme stress disorders not otherwise specified (DESNOS) This syndrome which was also called Complex posttraumatic stress disorder is composed of six groups of symptoms which are: I alteration of regulation of affect, II alteration in attention and consciousness, III alteration in self-perception, IV alteration in relationship with others, V somatization and VI alteration in system of meaning. Although this syndrome was first thought to occur at early age with repetitive traumatic events at context of care giving relationship then the concept was thought to be applicable to repetitive long duration traumas occurring within war situations or under totalitarian political regimes. ICD 10 on other hand described long living personality change upon long traumatic stress exposure. A recent conceptualization recognizes that more complex cases of PTSD involve deficits in regulating emotional distress that in addition to the core PTSD symptoms, complex presentations are more difficult to treat because they involve acting out, self-harm, and self-destructive relationships and behaviors. Although it was thought that complex trauma reactions can develop without necessity for presence of classical trauma syndrome yet recent empirical evidence found that only 8% of DESNOS patients don’t fulfill criteria of PTSD. For this reason complex PTSD was not included as separate disorder in DSM V while it was included in ICD 11 as disorder fulfilling criteria of both classical and complex trauma symptoms. From other perspective it is thought that cultural factors may have role in shaping reaction to psychological trauma and the application of Euro American view of this subject to traditional cultures need further evidence. The Iraqi Mental health survey found low rates of classical PTSD among Iraqi population (INHS 2007). Iraqi population sustained long and repetitive traumatic events through long war years and economic sanctions since 1980. The current study proposes that complex trauma reactions could have happened within Iraqi population and possibly overlooked if focus is on classical PTSD as trauma reaction within Iraqi culture. This study tries to examine the presence of complex trauma reaction (DESNOS) among Iraqi population.

Methodology

Design: cross sectional study arranged to examine complex PTSD construct in study subjects

Sample: Random sample was made of medical students at Al Nahrain University College of Medicine, Baghdad, Iraq from the academic year of 2018-2019. 131 students from fourth, fifth and sixth classes agreed to participate in the study and were provided with study forms. College adopts 6 years training system and total number of students within the last three classes was 450 students

Study tools: Study questionnaire form included three parts

§ Part one: demographics; this includes age, gender, marital status and level at medical school

§ Part two: include questions about any current or past psychiatric treatment, current physical disease and treatment. Participants who have such experiences were excluded from further analysis of trauma reaction.

§ Part three: questionnaire of past traumatic events. It examines the types and rates of variable psychological traumatic events. The questionnaire was already used as part of Iraqi National Mental Health Survey 2007 (INMHS 2007) It includes 27 questions to be answered by yes or no. Exposure to traumatic events was considered a precondition to be involved in further data analysis and 27 participants were excluded from data analysis, as they did not report any exposure to traumatic event while the other 127 were included as they reported such exposure

§ Part four: Complex trauma questionnaire: This self-rating questionnaire was based on the six groups of symptoms and criteria needed for DESNOS diagnosis. Questions were written according to criteria provided. The questionnaire included 45 items about the six symptom categories. Each of the six items includes variable number of questions to be answered according to the Likert questionnaire format. The 45 items of complex trauma part and the 27 items of the past trauma events part yield a final 72 items questionnaire for this study. The process of validity and reliability of the questionnaire were arranged according to the following steps.
Validity: The questionnaire was sent to 7 experts in psychology and psychiatry. Items which got 80% or more agreement among experts were kept as they are. Others needed simple changes according to notes of experts and the whole questionnaire was accepted as it is and was finally composed of 72 items.

Reliability: Test retest reliability was found to be of 0.79 while alpha Cronbach coefficient was 0.76 for the questionnaire.

Statistical analysis: SPSS software was used. Percentages, T test for independent variables, Pearson correlation coefficient, alpha Cronbach coefficient, T correlation equation were used at relevant data analysis process.

Results

1. Questionnaire: aim was accomplished and the final questionnaire is composed of 72 items; 27 being for traumatic events part while 45 items belong to complex trauma symptoms part. validity and reliability was confirmed as mentioned above.

2. Rates of traumatic events experience out of the 131 participants, 129 confirmed exposure to one or more traumatic event (98.4%). Data of these 129 participants were then further examined. The number of traumatic events was variable with one participant having 18 events and one other having 17 events. Rates of other traumatic events are further described in table 1.

Table 1 number of traumatic events for each participant (total 116)

<table>
<thead>
<tr>
<th>No. of events</th>
<th>No. of participants</th>
<th>No. of events</th>
<th>No. of participants</th>
<th>No. of events</th>
<th>No. of participants</th>
<th>No. of events</th>
<th>No. of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>6</td>
<td>16</td>
<td>10</td>
<td>11</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>7</td>
<td>10</td>
<td>11</td>
<td>5</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>8</td>
<td>11</td>
<td>12</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The most frequent event was being concurred, inspected by police, army or coalition forces being experienced by 65.5% of participants while sudden death of relative by killing or accident or heart attack was experienced by 62.9%. Table 2 summarizes the frequency for each event among study sample.

Table 2 frequency of each traumatic event

<table>
<thead>
<tr>
<th>Event item</th>
<th>No of subjects</th>
<th>%</th>
<th>Event item</th>
<th>No of subjects</th>
<th>%</th>
<th>Event item</th>
<th>No of subjects</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>0.414</td>
<td>10</td>
<td>47</td>
<td>0.405</td>
<td>19</td>
<td>5</td>
<td>0.043</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>0.112</td>
<td>11</td>
<td>5</td>
<td>0.043</td>
<td>20</td>
<td>12</td>
<td>0.103</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0.069</td>
<td>12</td>
<td>5</td>
<td>0.043</td>
<td>21</td>
<td>8</td>
<td>0.069</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>0.138</td>
<td>13</td>
<td>76</td>
<td>0.655</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.129</td>
<td>14</td>
<td>19</td>
<td>0.164</td>
<td>23</td>
<td>66</td>
<td>0.569</td>
</tr>
</tbody>
</table>
Complex trauma: Of the 129 participants who have experienced traumatic events, 13 were further excluded for presence of psychiatric history or being under psychiatric treatment or having chronic physical disease or treatment. 116 participants were further examined for complex trauma syndrome. As a whole 29 person (25%) of study sample fulfilled the criteria of DESNOS. Of these 73% confirmed the presence of criteria Criterion VI. Of complex trauma, (Alterations in Systems of Meaning), 39% confirmed the presence of criteria Criterion II (Alterations in Attention or Consciousness), 25.3% confirmed the presence of criterion IV. (Alterations in Relations with Others), 25.2% confirmed the presence of criterion V. (Somatization), 25.1% confirmed the presence of criterion I (Alteration in Regulation of Affect and Impulses), and 22.17% confirmed the presence of criterion III (Alterations in Self-Perception). (Table 3)

Table 3 frequency of each criterion of DESNOS

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>Criteria I.</th>
<th>Criteria II.</th>
<th>Criteria III.</th>
<th>Criteria IV.</th>
<th>Criteria V.</th>
<th>Criteria VI.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of degrees</td>
<td>151</td>
<td>78</td>
<td>133</td>
<td>46</td>
<td>126</td>
<td>146</td>
</tr>
<tr>
<td>The Level</td>
<td>25.1%</td>
<td>39%</td>
<td>22.17%</td>
<td>15.3%</td>
<td>25.2%</td>
<td>73%</td>
</tr>
</tbody>
</table>

Detailed statistical analysis revealed no significant relationships between rates of DESNOS and gender, marital status. (Tables 4, 5)

Table 4 rates of DESNOS according to gender (Pearson Chi square value 2.125, df 1, P value 0.145)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total</th>
<th>No DESNOS</th>
<th>Have DESNOS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>count</td>
<td>count</td>
<td>count</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>75</td>
<td>53</td>
<td>22</td>
<td>70.7</td>
</tr>
<tr>
<td>Female</td>
<td>41</td>
<td>34</td>
<td>7</td>
<td>82.9</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>87</td>
<td>29</td>
<td>75</td>
</tr>
</tbody>
</table>
Table 4: Rates of DESNOS according to marital status (Pearson Chi-Square value 0.066, df 1, P value 0.797)

<table>
<thead>
<tr>
<th></th>
<th>Total count</th>
<th>No CPTSD</th>
<th>Have CPTSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>count</td>
<td>%</td>
<td>count</td>
</tr>
<tr>
<td>Single</td>
<td>90</td>
<td>68</td>
<td>75.6</td>
</tr>
<tr>
<td>married</td>
<td>26</td>
<td>19</td>
<td>73.1</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>87</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Conclusions

1- The rate of DESNOS found in this study is high (25%). the Iraqi Mental Health Survey IMHS (2006-2007) found the life time and 12 month prevalence of classical PTSD to be 3.9 % and 1.6% within Iraqi society respectively. (12) Although the current study’s population is restricted to medical students and cannot be representative to whole Iraqi population but the high rate reported may indicate that complex trauma reaction can be wide spread. The current evidence shows that DESNOS is reported in variable rates among patients with classical PTSD (13) yet small percent of DESNOS do not suffer from the disorder. (14) This notion that complex trauma reactions occur in accompaniment with classical PTSD among traumatized populations is further prompted by evidence which support the validity of ICD 11 CPTSD as sibling yet separate disorder from PTSD. (15) The high rates of DESNOS as reported in this study in comparison to IMHS PTSD rates needs then to be explained from other perspective. Although the time interval between 2006 when IMHS was arranged and time of the current study was accompanied by continuous unstable circumstances in Iraq yet whether these circumstances lead to higher rates of PTSD this then need to be confirmed. DESNOS was considered a very useful construct by mental health professionals who have worked with adult non-Western patients exposed to forced migration or torture although there has been little research in this area. (16) The DESNOS construct raises questions about whether repeated exposure to trauma lead to a different pattern of symptoms than those included in PTSD and whether DESNOS model is useful in a cross-cultural context. (8) DESNOS emphasizes both dissociation and somatization, two symptoms not included in the DSM-IV PTSD diagnostic criteria that are frequently observed in traumatized non-Western cohorts? (17) The relevance of the PTSD diagnosis has been criticized from a cross-cultural perspective as a Euro-American construct that has little relevance to posttraumatic syndromes encountered in traditional societies. (18) Somatization and dissociation, two cardinal symptoms of posttraumatic reactions in traditional societies, are missing from DSM-IV diagnostic criteria for PTSD (but not DESNOS). (17) The current study shows that most of individuals in the sample had experienced several traumatic events and interestingly both dissociation and somatization are very highly reported. There may be culture-specific idioms of distress that provide a better characterization of posttraumatic distress syndromes found in one ethno cultural context or another. (19) The results of the current study suggest that DESNOS can be very common trauma reaction in Iraqi population. One limitation of the current study is that it did not examine possibility of occurrence of classical PTSD within studied sample. Further studies are needed on large-scale population samples to confirm whether DESNOS is common trauma reaction in Iraq and to conclude more whether DESNOS in Iraq occurs as separate diagnostic category regardless presence of classical PTSD syndrome. Findings from these studies can modify the attitude towards the impact of psychological trauma in Iraqi population. The low rates of PTSD in the IMHS were explained by possible inherent resilience present in Iraqi people. (20) If DESNOS is proved to be as such highly prevalent then we will need to modify our strategy towards impact of trauma at epidemiological, clinical, social and cultural levels. Improved detection
will help implement necessary treatment strategy that is different from that implemented in classical PTSD. (8)

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

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Revealing Potency of Bioactive Compounds as Inhibitor of Dengue Virus (DENV) NS2B/NS3 Protease from Sweet Potato (Ipomoea batatas L.) Leaves

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Abstract

This study aims to identify the potency of bioactive compounds of sweet potato leaf as inhibitory agent to dengue virus (DENV) NS2B/NS3 protease by using computational study. The 3D structure of NS2B/NS3 protease was collected from PDB and the 2D structure of several bioactive compounds of sweet potato leaf were obtained from PubChem. The visualization and data analysis were performed by using the PyMol software. According to the in silico analysis, result demonstrated that dehydroabietanol had the lowest free energy binding. However, based on the protein-ligand analysis, all the compounds showed the hydrogen bond and hydrophobic interaction. All the compounds with hydrogen bond could not be interacted with catalytic domain, but hydrophobic interaction could be interacted to the target domain via Ser135 by δ-Selinene and His51 by α-Caryophyllene. In summary, we conclude that δ-Selinene and α-Caryophyllene might have potencies as a therapeutically drug for dengue.

Keywords: Dengue virus, in silico, Ipomoea batatas, protease

Introduction

Indonesia is a tropical country and home of mosquito vector species of dengue virus (DENV), Aedes aegypti and Aedes albopictus[1]. DENV is infectious agent dengue fever-causing epidemic diseases, current antiviral drug cannot succeed against this entity. Therefore, the development of the drug is required to treat DENV infection[2]. DENV is member family from Flaviviridae and consist of four serotypes (DENV 1-4)[3].

Indonesia is a large country in Southeast Asia and has a high plant diversity in the world. There are more than 5,000 medicinal plants that available all around us[4]. Consequently, medicinal plants used by its population in curing many diseases[5,6]. The medicinal plants generate a variant of chemical composition with the potency to prevent viral replication and probable resource for controlling viral infection[7]. Plants have been described to have antiviral action and some have been accustomed to manage viral taints in humans and animals[8]. Medicinal plants were found for antiviral compounds, such as Convolvulaceae[9].

DENV is a single stranded and positive polarity RNA virus with a genome of about 11,000 bases in length. The genome encodes for three structural proteins and nonstructural proteins, its activated by trypsin-like NS2B/NS3 protease. This enzyme has a catalytic triad domain,
it contains a specific amino acid residual His51, Asp75, and Ser135 required for catalytic activity\[10\]. Protease complex NS2B/NS3 have a key role in viral replication and make it’s as a therapeutic target to development of protease inhibitor for DENV infection\[11\]. In addition, sweet potato bioactive compounds reported as a potent inhibitor of the virus\[12\]. Previous research by Pochapski et al. (2011) explained that chemical compound contained in sweet potato have potency as a drug but this mechanism action is unknown\[13\]. Therefore, we identified the potency of bioactive compounds of sweet potato leaf as inhibitory agent to DENV NS2B/NS3 protease by using computational study.

**Materials and Methods**

**Collection of NS2B/NS3 serine protease**

The target protein in this research is DENV NS2B/NS3 protease. Therefore, the 3D structure of NS2B/NS3 serine protease was obtained from protein database or RCSB with ID 2FOM. Furthermore, the protein was validated the model quality by using Ramachandran plot.

**Collection of sweet potato leaves compounds**

Various bioactive compounds from sweet potato leaves referred to previous research\[14\] were retrieved from PubChem. The 3D structure of compound has collected in structure data format (SDF). Therefore, it must be converted by Open Babel software to produce flexibility 3D structure with protein data bank (PDB) format.

**Molecular docking of NS2B/NS3 serine protease with sweet potato leaves compounds**

Screening the potency of sweet potato leaves compounds by using virtual screening was conducted with molecular docking. There are several methods which could be applied by using molecular docking, such as specific docking and blind docking\[15,16,17\]. This research was conducted by using blind docking by PyRx software to identify the potency of bioactive compound from sweet potato leaves as inhibitor NS2B/NS3.

**Visualization of interaction between NS2B/NS3 serine protease with sweet potato leaves compounds**

Then, we were using PyMol to visualize 3D ligand-protein structure of NS2B/NS3 serine protease and various compounds of sweet potato. Furthermore, those interaction were analyzed by using LigPlot to compared chemical interaction\[18,19\].

**Results and Discussion**

DENV is a single stranded and positive polarity RNA virus with a genome of about 11,000 bases in length. The genome encodes for three structural proteins and nonstructural proteins, its activated by trypsin-like NS2B/NS3 protease. This enzyme has a catalytic triad domain, it contains a specific amino acid residual His51, Asp75, and Ser135 required for catalytic activity\[10,11\]. Protease complex NS2B/NS3 have a key role in viral replication and make it’s as a therapeutic target to development of protease inhibitor for DENV infection.

The 3D structure has been obtained from PubChem, around thirty-six of a bioactive compound that’s contained in the leaf essential oil of sweet potato, then its minimize by Open Babel because then its minimize by Open Babel because it’s will making this ligand have lowest binding energy and generate structure flexibility NS2B/NS3 serine protease (2FOM) obtain from PDB, and then validating structure has been done using RAMPAGE, the result of structure validation showed by Ramachandran plot. Structure validation aims to evaluate the structure quality of targeted protein which is quantify by the number of favored amino acid that reach more than 90%\[19\]. In this study, targeted protein was visualized based on surfaces structure. Furthermore, the NS2B/NS3 structure is arranged from two chain, A and B which contain α-helix, β-sheet, and coil.
Molecular docking is a part of in silico analysis that has main objective to identify the energy binding size and the interaction pattern between protein and its ligand according to the chemical interaction within the complex\[^{[16]}\]. Molecular docking has performed by PyRx software to know binding affinity level with grid positions x: -0.4637 y: -15.1662 z: 16.1087 and dimensions (Å) x: 52.1952 y: 56.6277 z: 46.8960 refer to catalytic site domain on NS2B/NS3 serine protease with amino acid residual is His51, Asp75, and Ser135, that be visualized by PyMol software as stick in cartoon structure with surface on target protein (Figure 1). After we know the positions of the catalytic site, then docking grid was directed to its. Docking result indicated that compound has a lowest binding affinity is dehydroabietinol around -7.1 kcal/mol. We revealed another results, such as abiadiene (-6.9 kcal/mol), cembrene (-6.6 kcal/mol), δ-selinene (-6.5 kcal/mol), δ-cadinene (-6.4 kcal/mol), spathulenol (-6.2 kcal/mol), β-euvebeene (-6.0 kcal/mol), γ-gurjunene (-5.9 kcal/mol), α-bergamotenol (-5.8 kcal/mol), β-caryophyllene (-5.8 kcal/mol), β-elemene (-5.8 kcal/mol), allo-aromadendrene (-5.7 kcal/mol), β-chamigrene (-5.7 kcal/mol), β-panasinsene (-5.7 kcal/mol), trans-α-bergamotene (-5.7 kcal/mol), caryophyllene oxide (-5.6 kcal/mol), γ-elemene (-5.6 kcal/mol), longifolene (-5.6 kcal/mol), α-caryophyllene (-5.5 kcal/mol), α-thujene (-5.5 kcal/mol), octadecanoic acid (-5.5 kcal/mol), bicyclogermacrene (-5.4 kcal/mol), limonene (-5.3 kcal/mol), terpinen-4-ol (-5.3 kcal/mol), eugenol (-5.2 kcal/mol), n-hexadecanoic acid (-5.2 kcal/mol), α-farnesene (-5.1 kcal/mol), α-santalol (-5.1 kcal/mol), γ-terpinene (-5.1 kcal/mol), p-cymene (-5.1 kcal/mol), p-menth-1-ene (-5.1 kcal/mol), bicycloelemene (-5.0 kcal/mol), α-pinene (-4.8 kcal/mol), β-pinene (-4.8 kcal/mol), cis-sabine (-4.8 kcal/mol), and 1-octen-3-Ol (-4.5 kcal/mol). Binding affinity is energy bonding formed from the interactions some molecule with other, several parameters affect binding affinity such as amino acid residues and type of chemical interaction between ligand-protein such hydrogen, hydrophobic, and Van der Waals. Binding affinity commonly used as indicator of binding energy to determine the complex interaction which is considering as biological activity outcome\[^{[16]}\].
Based on results of molecular docking, a bioactive compound from sweet potato leaves have a potency to bind NS2B/NS3 with lowest binding affinity and possible formed stable complex of ligand-protein. The amount of free energy (ΔG) indicating as an ability of binding for a bioactive compound to the target protein. In this condition, the ligand bind to target protein and making of energy alteration such Gibbs free energy (ΔG), it has negative value when the system in equilibrium condition with constant pressure and temperature, because the widely of protein-ligand association can be determined by negative ΔG, so its determination of the protein-ligand complex or ligand binding affinity\[20\].

Figure 2. Targeted domain and δ-Selinene interaction. The ligand was shown as yellow stick structure, while the specific residual amino acid was shown as red color. The hydrophobic interaction was occurring in Ser135 area shown in black circle.

Figure 3. The α-Caryophyllene interaction to targeted domain. The ligand was shown as purple stick structure, while the red color is the residual amino acid in NS2B/NS3. The hydrophobic interaction was occurring in Ser135 which shown inside the black circle.
Potency as inhibitor NS2/NS3 serine protease in the oil essential of sweet potato leaves showing dehydroabietinol have lowest free energy binding, but refer to the result of analysis of protein-ligand domain interaction of the all compound showing, it’s had two type of chemical interaction between ligand with protein domain as hydrogen bond and hydrophobic. All of the compounds have hydrogen bond cannot be interacted with catalytic domain, but hydrophobic interaction can be interacted to target domain, via Ser135 by δ-Selinene (Figure 2) and His51 by α-Caryophyllene (Figure 3). Catalytic triad (Ser135, His51, and Asp75) can be found in NS2B/NS3 and required to protease activity which functions as a mechanism for activation of DENV replication\(^{[11]}\). So, it is predicted that the two compounds are very possible to interact with the catalytic site domain in NS2B/NS3. Therefore, it can be potentially as an inhibitor compare to dehydroabietinol which has lower binding affinity without chemical interaction site in targeted domain in NS2/NS3 serine protease.

**Conclusion**

In summary, the δ-Selinene and α-Caryophyllene are predicted to have potency as dengue disease medication through the inhibitory mechanism against DENV. Specifically, the ligands are binding to one of catalytic triad of residual amino acid. This interaction inhibits the targeted protein activation when the virus replication occurs.

**Conflict of Interest:** The author declare that they have no conflict of interest.

**Funding:** This study supported by the Generasi Biologi Indonesia Foundation, Indonesia.

**Acknowledgements:** We would like to declare our sympathy to the victims of COVID-19. Tribute goes to the frontliners worldwide, especially in Indonesia. We thank EJA, Indonesia for editing the manuscript.

**Ethical Approval:** No ethical approval needed.

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The Role of EMMPRIN in Perirenal Fat Invasion Clear Cell Renal Cell Carcinoma

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Abstract

Renal Cell Carcinoma (RCC) is a common malignancy of the kidney, and Clear Cell Renal Cell Carcinoma (CCRCC) is the most common type. Accurate prediction of prognosis is valuable for therapy and follow up. EMMPRIN is a transmembrane protein of the immunoglobulin family and is associated with tumour proliferation, invasion and metastasis. This study aims to prove the role of EMMPRIN in perirenal fat invasion CCRCC. This analytic observational study with a cross-sectional approach conducted in Anatomical Pathology Institute of Dr. Soetomo Hospital, Surabaya that used 44 samples of paraffin blocks from radical nephrectomy preparations for CCRCC patients at the period of January 2013-December 2018, which were divided based on perirenal fat invasion status. The analyzed was using Spearman test. EMMPRIN expression is positively correlated with perirenal fat invasiveness (\( p = 0.019 \)) in clear cell renal cell carcinoma. EMMPRIN expression is related with perirenal fat invasiveness, in clear cell renal cell carcinoma.

**Keywords:** Clear cell renal cell carcinoma, EMMPRIN, perirenal fat invasion

Introduction

The Renal Cell Carcinoma (RCC) is the sixth most common malignancy diagnosed in men and tenth in women and accounts for approximately 85% of malignant kidney tumors and 2% of all malignant tumors. In Europe and North America, the lifetime risk for developing renal cell carcinoma ranges between 1.3% and 1.8%. According to the latest data from the World Health Organization (WHO), there are more than 140,000 deaths related to renal cell carcinoma each year, with renal cell carcinoma ranking as the 13th most common cause of cancer death worldwide.¹,²

Tumor staging (TNM), defined by the disease’s anatomical involvement, is recognized as one of the strongest prognostic factors in the clinical outcome of patients with RCC, as described in the eighth edition of the American Joint Commission on Cancer (AJCC) Cancer Staging Manual. The system currently used is according to the 2016 WHO classification system. This system concerns tumor size, tumor growth through the renal capsule, tumor invasion of the renal veins, lymph nodes, and metastases to the adrenals, and distant metastases. These factors indicate a poor prognosis compared to tumors confined to the kidney.³,⁴

EMMPRIN/CD147, also known as Basigin (BSG) or Extracellular Matrix Metalloproteinase Inducer (EMMPRIN), is a transmembrane glycoprotein belonging to the immunoglobulin superfamily that is highly expressed on the cell surface of various types of tumors, including breast, lung, mouth, esophageal cancer, larynx and kidney.⁵ EMMPRIN expression was positively expressed by 88.7% in advanced RCC.⁶ EMMPRIN is known to induce the production of various Matrix Metalloproteinases (MMPs) in cancer cells and

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fibroblasts after epithelial-stromal interactions. MMP is a major protease in degrading the extracellular matrix, which leads to cancer cell invasion and metastasis.⁷

EMMPRIN binds to Cyclophilin A (CypA). A previous study showed that the CypA-EMMPRIN interaction-initiated growth was signaling via a variety of pathways, including the MAPK, ERK1/2, and p38 signaling pathways that induce G1 to S transitions via cyclin D1 and p-RB in cholangiocarcinoma.⁸

Correlation between EMMPRIN expression in cell carcinoma kidney has not been reported. Therefore, this study was conducted to analyze EMMPRIN expression with perirenal fat invasion status in renal cell carcinoma. This study aims to prove the role of EMMPRIN on the status of perirenal fat invasion in Clear Cell Renal Cell Carcinoma (CCRCC).

**Materials and Methods**

**Preparation and Sample of the Study**

This study’s research design was an analytic observational study with a cross-sectional approach, which was carried out in the Anatomic Pathology Installation of Dr. Soetomo General Academic Hospital Surabaya, Indonesia. The study sample used 44 blocks of paraffin radical nephrectomy preparations for CCRCC patients at the Anatomical Pathology Institute of Dr. Soetomo General Academic Hospital, Surabaya, for the period January 2013-December 2018. The samples were divided into two groups based on perirenal fat invasion status. The parameter of assessment was the expression of EMMPRIN, which streaked positively on tumor cells. This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ethical Clearance No.1705 / KEPK / XII / 2019).

**Immunohistochemical Procedures**

EMMPRIN expression in samples was observed using immunohistochemical staining. Paraffin blocks were cut 4 µm, deparaffinized, and rehydrated with graded alcohol, then warmed with citrate buffer pH 6 for 20 minutes in the microwave. The primary antibody, namely EMMPRIN (sc-71038, Santa Cruz Biotechnology, Inc.), was dripped by diluting 1: 250 at 40°C overnight. The secondary antibody is then dropped and incubated for 20 minutes. The final step, diaminobenzidine (DAB), was dripped, and counterstain was carried out with Meyer Hematoxylin.

**Immunohistochemical Staining Analysis**

EMMPRIN expression was assessed using an Immunoreactive Score (IRS), which is the multiplication of the percentage of tumor cells stained (A) and the intensity of staining (B). The percentage is divided into a score of 0 = no positive tumor cells, score 1 = positive tumor cells < 10%, score 2 = positive tumor cells 10 - 50%, score 3 = positive tumor cells 51 - 80%, and score 4 = cells positive tumors > 80%. Intensity was divided into a score of 0 = colorless, score 1 = weak intensity, score 2 = moderate intensity, and score 3 = strong intensity. The IRS (AXB) was divided into four groups, namely negative (score 0), weak (score 1 - 3), moderate (score 4 - 8), and strong (score 9 - 12). EMMPRIN expression was observed in the membrane and cytoplasm of tumor cells.⁹ EMMPRIN expression was observed using a binocular light microscope and evaluated by two pathologists.

**Statistical Analysis**

The correlation between perirenal fat invasion status and EMMPRIN expression was tested by the Spearman correlation test. The test results are said to have a significant correlation if the *p*-value is <0.05.

**Results and Discussion**

The patients’ average age was 53.89 years with a male to female ratio of 2:1. In this study, clear cell, non-perirenal fat invasive (non-PFI) renal cell carcinoma was found in 59.1% (26/44) of cases, whereas clear cell, perirenal fat invasive (PFI) type renal cell carcinoma was only 40, 9% (18/44) of cases. The highest grade in this study was grade 3 (54.5%) cases (24/44). The clinicopathological characteristics of the patients are shown in Table 1.
Table 1. The clinicopathological characteristics of the patient.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) #</td>
<td></td>
</tr>
<tr>
<td>£ 40</td>
<td>2 (4.6)</td>
</tr>
<tr>
<td>41-50</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td>51-60</td>
<td>17 (38.6)</td>
</tr>
<tr>
<td>61-70</td>
<td>11 (25.0)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (72.7)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (27.3)</td>
</tr>
<tr>
<td>Tumor Grade</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>5 (11.4)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>12 (27.3)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>24 (54.5)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>Perirenal Fat Invasion</td>
<td></td>
</tr>
<tr>
<td>(pT1-2) / Non PFI</td>
<td>26 (59.1)</td>
</tr>
<tr>
<td>(pT2-4) / PFI</td>
<td>18 (40.9)</td>
</tr>
</tbody>
</table>

EMMPRIN expression in this study was stained on the membrane and cytoplasm of tumor cells (Figure 1). The results of this study indicated that EMMPRIN expression with a strong IRS score was more common in clear cell renal cell carcinoma with perirenal fat invasion, namely 66.7% (Table 2). The Spearman correlation test results showed a significant correlation between perirenal fat invasion status and EMMPRIN expression ($p < 0.05$) with a value of $r = 0.352$ (Table 3). These results indicate that the higher the EMMPRIN expression is in line with the perirenal fat invasion status.

Figure 1. EMMPRIN expression by immunohistochemical staining on clear cell renal cell carcinoma, magnification: 400×. A: EMMPRIN expression with weak intensity; B: Medium intensity EMMPRIN expression; C: EMMPRIN expression with strong intensity.
Table 2. EMMPRIN expression on the status of perirenal fat invasion of Clear Cell renal cell carcinoma.

<table>
<thead>
<tr>
<th>EMMPRIN Expression</th>
<th>Category</th>
<th>Non-invasive perirenal fat</th>
<th>Invasive perirenal fat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage</td>
<td>&lt;10%</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-50%</td>
<td>1 (3.8%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51-80%</td>
<td>21 (80.8%)</td>
<td>11 (61.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;80%</td>
<td>4 (15.4%)</td>
<td>7 (38.9%)</td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>Weak</td>
<td>3 (11.5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>14 (53.9%)</td>
<td>7 (38.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td>9 (34.6%)</td>
<td>11 (61.1%)</td>
<td></td>
</tr>
<tr>
<td>IRS Score</td>
<td>Weak</td>
<td>4 (15.4%)</td>
<td>0 (0%)</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>13 (50%)</td>
<td>6 (33.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td>9 (34.6%)</td>
<td>12 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. The Spearman correlation test results of EMMPRIN expression with perirenal fat invasion status.

<table>
<thead>
<tr>
<th>Perirenal fat invasion status</th>
<th>EMMPRIN Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.352</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

Most of the patients in this study were in the age range 51 - 60 years with 17 cases (38.6%), with a male to female ratio of 2:1 where the number of male cases was 32 cases (72.7%). This is in accordance with previous research which states that the highest incidence is found in the sixth and seventh decades of life and about 80% are between the ages of 40 - 69 years with the distribution of men more than women. The results of this study are in line with the research conducted by Zheng et al., which stated that the increased expression of EMMPRIN was significantly correlated with tumor size, depth of invasion, lymph vessel invasion, MMP 2, MMP 9, and tumor VEGF ($p < 0.05$) in gastric carcinoma. This study showed that the abnormal expression of EMMPRIN can increase tumor cell invasion and angiogenesis by increasing the expression of MMP and VEGF in stromal fibroblasts and gastric carcinoma cells so that increased EMMPRIN expression could be used as an effective and objective marker in predicting invasion and prognosis in gastric carcinoma. A study by Nakamura et al. also found that high EMMPRIN expression is a significant marker of poor prognosis in endometrial cancer. EMMPRIN affects the proliferation, migration, and invasion of tumor cells.
through the expression of TGF-β, EGF, VEGF, MMP-2, MMP-9. The binding between growth factors such as TGF beta, EGF, IGF, and TNF alpha with their receptors can activate cadherin E inhibiting factors such as Snail. The decrease in E-cadherin expression resulting in the loss of bonds between cells is an early stage of EMT.13

Various studies have shown that EMMPRIN plays an important role in the invasion and metastasis of various tumors, such as hepatocellular carcinoma, astrocytic glioma, retinoblastoma, and oral squamous cell carcinoma through increased MMP production. EMMPRIN has also been found to play a role in urothelial carcinoma invasion through the secretion of MMP2, MMP9, MMP14, and VEGF.14

The multifunctional role of EMMPRIN in advanced RCC is not only as an adhesion molecule involved in Cell-Matrix-Extracellular interactions (ECM) but also as a mediator for tumor invasion and angiogenesis through stimulation of VEGF production. Multivariate analysis showed a strong association between EMMPRIN and VEGF expression and poor prognosis in advanced RCC.6 EMMPRIN expression was found to be significantly associated with increased tumor invasion. These observations strongly suggest that EMMPRIN may be actively involved in the growth, invasion, and metastasis of OSCC. In addition, measurement of EMMPRIN levels can help predict a patient’s prognosis.15

EMMPRIN is a transmembrane glycoprotein belonging to the immunoglobulin superfamily that is highly expressed on the cell surface of various types of tumors, including kidney cancer.5 EMMPRIN acts as a cellular adhesion molecule and induces the secretion of matrix metalloproteinases (MMPs) and the release of cytokines.16 EMMPRIN stimulates cancer cells and fibroblasts peritumoral to secrete matrix metalloproteinases (MMPs), which are capable of lowering extracellular matrix protein (ECM), and EMMPRIN directly promotes tumor proliferation, invasion, and metastasis.17

EMMPRIN has been shown to be involved in the regulation of tumor cell invasion and metastasis. First, EMMPRIN combines with the alpha6beta1 integrin into the FAK P13K-Ca (2+) pathway and the MARK signal, which then produces interstitial collagenase (MMP-1), forming a CD147-MMP-1 complex on the surface of tumor cells, thus modifying the pericellular cell matrix tumor to promote invasion. Second, EMMPRIN is a receptor for platelet GPVI and mediates platelet movement through the GPVI-EMMPRIN Combination, thereby increasing the potential for metastasis. High EMMPRIN expression can be used to determine the TNM stage, histopathological stage, metastases, and worse survival in patients with kidney cancer.18

Another study investigated the effects of EMMPRIN on prostate cancer proliferation. EMMPRIN is expressed on the cell surface of most tumor cells, which results in proliferation, invasion, metastasis, and angiogenesis of cancer cells. Previous studies have shown that EMMPRIN can increase prostate cancer invasion and metastasis. The study showed that the inhibition of the EMMPRIN gene had a significant effect on the prostate cancer cell cycle, where a decrease in EMMPRIN expression resulted in an increase in the G0/G1 phase and a significant decrease in the S and G2 phases, indicating the cessation of the G1 phase. The G1 phase, the cell cycle phase in which cells grow and synthesize mRNA and protein for DNA synthesis, is very important because it determines whether the cell is committed to division or escape the cell cycle. The study states that EMMPRIN suppresses the progression of cancer cells by resting the cell cycle in the G0/G1 phase of cancer by suppressing cyclin D1 expression, thereby inhibiting cell proliferation.19,20

**Conclusion**

In conclusion, EMMPRIN expression was significantly correlated to the perirenal fat invasion. EMMPRIN expression has an important role in the Clear Cell Renal Cell Carcinoma (CCRCC).

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Acknowledgements:** The author would like to express the gratitude to all lecturers in Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. We thank Dr. Budi Utomo, dr., M.Kes. for statistical analysis. We additionally thank Arif Nur Muhammad Ansori for editing the manuscript.
Ethical Approval: This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ethical Clearance No. 1705/KEPK/XII2019).

Source of Funding: Self-funding research.

References


Measuring the Health Impact of Drinking Water Sources in El-Fashir, Sudan

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Abstract

The study aimed to assess the physical and chemical condition of drinking water in El-Fashir city, Sudan. The parameters studied are; Electrical conductivity (EC), nitrates, pH, Total Dissolved Solid (TDS) and fluorine were analyzed at water points and in the laboratory. Results were processed on SPSS software. The study revealed the pH results, which were distributed between 6.8 to 8 for samples from hand pumps 1 and 2 and tank water, respectively. The residual chlorine concentration (RC) in the water supply system of the selected stations ranged between 0.2 to 0.6 mg /L. The concentration of nitrate level ranged from 3.4.5 mg/L, which is within the acceptable range of the standards for WHO and SSMO standards. The study also determined the level of fluorine, which ranged between 0.17 - 0.41 mg/L, which is within the acceptable range of the standards for WHO and SSMO standards. This study found that all results fall within the permissible limits of the standards of WHO and SSMO standards except the hand pumps.

Keywords— Water Quality, Drinking Water, TDS, Nitrate, Conductivity, Turbidity, Fluoride.

Introduction

Water is one of the most vital of all-natural resources known on earth. It is essential to all living organisms, human health, food production, most ecological systems and economic development (¹). The safety of drinking water is vital for the health issues. The safety of drinking water is affected by numerous impurities, which included physical, chemical and microbiological. Such impurities cause serious health problems (²). Water is vital to maintain life, and it must be accessible, safe, adequate and available. Improving the accessibility to safe drinking water can result in appreciable benefits for human health. Major efforts should be made to achieve high quality drinking-water (²,³).

Water covers 75% of our planet, yet, only a small fraction is available as fresh water. Where the majority of water (97%) is found in the oceans (too salty), the remaining 3% of water is fresh where 99.9 % of this water is locked up in the poles, or is so deep (buried) underground that it is too costly to extract (¹).

Sources of Water categories of naturally occurring water resources as groundwater, dug wells, borehole wells, spring water, rainwater and surface water. Potable or drinking water “defined as the water delivered to the consumer that can be safely used for drinking and domestic’s purposes”. In Sudan, the main sources of water are ground water, surface water (rivers and streams) depending heavily on the rainfall. Furthermore,
the clean water supplies are managed by specific water cooperation, and the quality of the water is monitored by authorities (3).

Access to water supply is an essential need and a human right. Joint Monitoring Programmers for water supply and sanitation stated that billions of people worldwide are lasting to suffer from poor access to water, sanitation and hygiene and according to UNICEF and the World Health Organization (WHO) reports (4). One of the targets of Sustainable Development Goal (SDG) 6.1, which is concerned mainly with water supply, “is to achieve universal and equitable access to safe and affordable drinking water for all by 2030” (5–7). Water quality considered safe and fit for human consumption if all parameters being in standards and according to national and international guidelines as explained in the bellow table1, which expressed the standards for selected parameters include among others EC, pH, R. Chlorine, Turbidity, TDS, Nitrate ……. etc. These parameters can affect the drinking water quality if the concentrations higher than the safe limits seted by the World Health Organization (WHO) and SSMO (8).

During the last years, it has been observed that water sources were exposed to pollution due to the increasing urbanization and human activities. These pollutants are classified as organics, inorganics, radionuclides, disinfectants and microorganisms (4). The inorganic chemicals are the major pollutant in drinking water (5). Heavy metals accumulate in human organs and nervous system which hinders the normal functions where it causes health problems (2). Moreover, the heart diseases, kidney-related problems, and cancer are related to the traces of metals such as chromium (Cr) and cadmium (Cd) reported as water-related diseases (6). Fluoride can have an adverse effect on tooth enamel and may give rise to mild dental fluorosis. In general, dental fluorosis does not occur in temperate areas at concentrations below 1.5–2 mg/L of drinking-water (9).

The present study aimed to assess the physio-chemical characteristics of water samples collected from diverse drinking water sources in the study area El-Fashir Sudan, and to compare the results obtained with the local (national) and international standards and guidelines.

Material and Methods

Study area description

The research was conducted in El-fashir city “which ‌is the capital city of North Darfur, Sudan” (1). The geographic coordinates are Latitude: 13° 37’ 40” N and Longitude: 25° 20’ 57” E Lat/Long (dec): 13.62793,25.34936 Köppen. With an estimated population of over 500,000 people, (2013), It considered as the capital city as while as the economic hub of North Darfur. and an agricultural marketing point in the surrounding region.

Sampling and analytical procedure

The samples were collected during August 2017, from 7 sources, such as tap (piped) water, tanks water, tape-stand and Hand pumps used for domestic purposes. Samples were treated using standard methods (collected, stored and transferred to the laboratory). The collection was in clean screw caped glass bottle, insulated ice chest with ice packs was used from the collection of samples. The collected samples were labelled with date and code and kept at 4°C until analyzed. Immediately after sample collection, taste, turbidity, temperature, pH, conductivity and TDS, Residual chlorine, were tested on sites. Nitrate and Fluorine were analyzed at the lab. The physio-chemical parameters were determined according the Standard Methods for the Examination of Water WHO and SSMO. Then comparing the results with SSMO and WHO.

Determination of Physio-chemical Parameters

- Analysis of pH levels: the measurement of pH was done on site by using a portable pH meter. Calibration was by standard buffer solutions; pH= 4 and 7, with temperature adjustments.
- Conductivity and TDS were carried out at 25°C by conductivity meter (Jenway 470) after calibration with calibration solution.
- Determination of Turbidity: Turbidity was measured using Palin test Portable Turbidity Meter.
- Free Chlorine measured by using color comparator; with added one DPD No 3 tablet, crushed and mixed to dissolve, and then stands for few minutes allowing full colour development; Free chlorine reacts
with diethyl-p-phenylene diamine (DPD) in buffered solution to produce a pink coloration. The intensity of the colour is proportional to the free chlorine concentration, then the reading was taken in the usual manner.

- Nitrate and Fluoride concentration: Nitrate and Fluoride values were determined by using spectrophotometer 7500. Procedure is mentioned in Chen et al., 2016 \((^{10})\).

### Table 1: Physio-chemical parameters standards for WHO and SSMO.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Items</th>
<th>SSMO standards mg/L</th>
<th>Standard specifications according to the World Health Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Concentration Permitted mg/liter</td>
</tr>
<tr>
<td>1</td>
<td>Color</td>
<td>Colorless</td>
<td>Colorless</td>
</tr>
<tr>
<td>2</td>
<td>Taste</td>
<td>Palatable</td>
<td>Palatable</td>
</tr>
<tr>
<td>3</td>
<td>Smell</td>
<td>Palatable</td>
<td>Palatable</td>
</tr>
<tr>
<td>4</td>
<td>EC</td>
<td>&lt;1400 μS/cm</td>
<td>&lt;1400 μS/cm</td>
</tr>
<tr>
<td>5</td>
<td>pH</td>
<td>6.5-8.5</td>
<td>6.5-8.5</td>
</tr>
<tr>
<td>6</td>
<td>R. Chlorine</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>Turbidity</td>
<td>5 NTU</td>
<td>&lt;5 NTU</td>
</tr>
<tr>
<td>8</td>
<td>TDS</td>
<td>&lt;300 mg/L</td>
<td>300 mg/L</td>
</tr>
<tr>
<td>9</td>
<td>Temperature</td>
<td>25oC</td>
<td>25oC</td>
</tr>
<tr>
<td>10</td>
<td>Nitrate</td>
<td>10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>11</td>
<td>Fluoride</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Results & Discussion

The results of water quality analysis for the various samples collected from different sources of water are shown in the table 2. The results are illustrated in table 2. pH values were all the samples ranged between 6.8 and 8, the lowest and the highest values are from samples (hand Pumps 1&2) and (tank water), respectively. According WHO and NDWQS pH range is between 6.8 and 8.5 which consistent with our results (Table 1). This result is consistent with the study conducted in the State of Perak, Malaysia the pH values ranged between 7.01 and 8.21 \((^{8})\). pH is classified as the most essential parameter of water quality; pH measurement is related to the alkalinity or acidity of water. Acidic water causes corrosion of the metallic plumbing system and pipes. Whereas, alkaline water shows disinfection properties \((^{11})\).
### Table 2: Laboratory Test Results

<table>
<thead>
<tr>
<th>S/N</th>
<th>Sources</th>
<th>EC (μs/cm)</th>
<th>pH</th>
<th>R. Chlorine (mg/l)</th>
<th>Turbidity (NTU)</th>
<th>TDS (mg/l)</th>
<th>Temperature</th>
<th>Nitrate (mg/l)</th>
<th>Fluoride (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tap 1 (Public Net)</td>
<td>440</td>
<td>7.8</td>
<td>0.6</td>
<td>1</td>
<td>570</td>
<td>26</td>
<td>3</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td>Hand Pump 1</td>
<td>2130</td>
<td>6.8</td>
<td>0</td>
<td>2</td>
<td>1491</td>
<td>27</td>
<td>4.1</td>
<td>0.17</td>
</tr>
<tr>
<td>3</td>
<td>Tape-stand</td>
<td>484</td>
<td>7.6</td>
<td>0.2</td>
<td>1.5</td>
<td>577</td>
<td>26</td>
<td>4.1</td>
<td>0.17</td>
</tr>
<tr>
<td>4</td>
<td>Tank</td>
<td>814</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>570</td>
<td>26</td>
<td>3.5</td>
<td>0.41</td>
</tr>
<tr>
<td>5</td>
<td>Tap 2 (Public Net)</td>
<td>440</td>
<td>7.8</td>
<td>0.6</td>
<td>1</td>
<td>566</td>
<td>25</td>
<td>3</td>
<td>0.41</td>
</tr>
<tr>
<td>6</td>
<td>Tap 3</td>
<td>440</td>
<td>7.8</td>
<td>0.5</td>
<td>1</td>
<td>550</td>
<td>28</td>
<td>3</td>
<td>0.41</td>
</tr>
<tr>
<td>7</td>
<td>Hand Pump 2</td>
<td>2130</td>
<td>6.8</td>
<td>0</td>
<td>2</td>
<td>1495</td>
<td>27</td>
<td>4.5</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The study showed the concentrations of residual chlorine (RC) in the study area water supply system for the selected stations which were varied between 0.2 to 0.6 mg/L. Most RC concentrations of these hand pumps and ground tanks were zero, while the tap-stand was 0.2 mg/L which is less than acceptable limits in WHO, and in the tap of the public net was 0.6 mg/L which is higher than acceptable limits in WHO. In general, the concentrations of Residual Chlorine are likely to be less in the far distance points from Water Supply Plant (WSP) comparing with near points to WSP. According to WHO, municipal potable water supplies are usually chlorinated to provide a residual concentration between 0.5 to 2.0 ppm to provide continual protection throughout the distribution system from the treatment plant to consumer taps (12). Figure 1 shows a comparison between RC and pH for different water sources in El-fashir.

![Figure 1 Comparison between RC and pH for different water sources in El-fashir.](image-url)
The present study pointed out the level of Electrical Conductivity represents the number of dissolved salts in the water. The presence of dissolved solids included chloride, calcium, and magnesium in water samples to enhance carrying the electric current. According to NDWQ, the maximum acceptable level of conductivity is 1000 $\mu$S/cm. The results ranged from 440 $\mu$S/cm to 2130 $\mu$S/cm, and the average conductivity value is 982.6 $\mu$S/cm (Table 3). The lowest and highest conductivity values correspond to Hand Pumps 1&2 and Public net samples, respectively.

It is expected to find high mineral contents in mineral water, resulting in higher conductivity values. The hand Pumps values cross the acceptable limit in WHO but still within acceptable limits in other references. Conductivity does not have a direct impact on human health but high conductivity affects the quality of the water by giving mineral taste to the water (2,8). Figure 1 shows the Comparison between RC and pH for different water sources in El-fashir.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>982.5714</td>
<td>795.12323</td>
</tr>
<tr>
<td>pH</td>
<td>7.5143</td>
<td>0.50143</td>
</tr>
<tr>
<td>Free Residual Chlorine</td>
<td>0.286</td>
<td>0.3024</td>
</tr>
<tr>
<td>Turbidity</td>
<td>1.500</td>
<td>0.5000</td>
</tr>
<tr>
<td>TDS</td>
<td>830.571</td>
<td>450.8924</td>
</tr>
<tr>
<td>Temperature</td>
<td>26.4286</td>
<td>0.97590</td>
</tr>
<tr>
<td>Nitrate</td>
<td>3.54286</td>
<td>0.550325</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.3071</td>
<td>0.12829</td>
</tr>
</tbody>
</table>

Total Dissolved Solids (TDS): contains mainly of inorganic salts such as carbonates, bicarbonates, sulphates, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron and a small quantity of organic matter diluted in water. The values of TDS ranged from 550 to 1495mg/L. The levels of TDS exceeded the allowed lowest limit recommended by WHO and SSMO (< 300 mg/L), where the hand pumps samples exceed the highest allowed limit (1000 mg/L) recommended by WHO and SSMO for drinking water. The mean total dissolved solids concentration in the study area was found to be 830.6 mg/L. The concentration of TDS in the present study was in contrast to the results found in the study conducted in Ethiopia where it ranged between 114.7 and 121.2 mg/L. The mean total dissolved solids concentration in the Wondo Genet campus was found to be 118.19 mg/l, which is within the limits of WHO standards (13). High values of TDS in groundwater are generally not dangerous to human health, but the high values of TDS may affect people health who are suffering from chronic kidney diseases, heart diseases and may cause laxative or constipation effects (13,14). The finding indicates that the relation between EC and TDS in drinking water was statistically significant, p <0.000.
The level of turbidity shown in Table 2. Turbidity is related to the cloudiness of water caused by a variety of particles. All the results below the maximum standard limit set by WHO and SSMO (5). Nephelometric Turbidity Units (NTU), where the lowest value was 1 NTU, and the highest value was 2 NTU which represents the samples from taps and hand pumps. These results were consistent with the result observed from the study conducted in Jordan where the turbidity value ranged between 0.05–4.8 NTU (15). High-quality drinking water must have a low level of turbidity to maintain human health. The study also revealed the level of Nitrate in raw water, and mainly it is a form of N₂ (of its oxidizing state). The values were 3 - 4.5 mg/L it is in the range of acceptable levels of WHO and SSMO standards (10 mg/L). Nitrates in water indicate the presence of entirely oxidized organic matter. The excess level of nitrates can cause Methemoglobinemia as a blue baby disease. Although nitrates levels that affect infants do not posture a direct threat to older children and adult people, they do indicate the potential presence of other more serious residential or agricultural pollutants such as bacteria or pesticides (16). The study also pointed out the level of fluoride the values ranged between 0.17-0.41 mg/L (mean= 0.31±0.12 mg/L), it is in the range of acceptable levels of WHO and SSMO standards (1.5mg/L). This result consisted of the results of a survey conducted in Behshar City the values ranged from 0.12 to 0.39 mg/L (mean 0.25±0,06 mg/L) (17). Figure 2 shows the exceedance values for Nitrate and Fluoride above WHO limit for water samples from El-fashir.

![Figure 2 Exceedance values for Nitrate and Fluoride above WHO limit for water samples from El-fashir](image)

**Conclusion**

The levels of water quality parameters such as pH, temperature, turbidity, fluoride and nitrate were found for all samples collected from different sites in the city of El-fashir within the limits recommended by WHO and SSMO, while the conductivity and TDS were found to be at the acceptable levels in all locations except for hand pumps were it exceed the recommended limits. It is also important to conduct further tests to check for other potential water contaminants such as chemicals, microbial and radioactive materials to complete the study on water quality at El-fashir city.

**Declaration of Competing Interest**: The authors declare that they have no conflicts of interest to disclose.

**Funding**: there is no financial support.

**Ethical Clearance**: Taken from State Ministry of Health.

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7. UN GGIM. Final list of proposed SDG. Rep Inter-Agency Expert Gr Sustain Dev Goal Indic. 2016;Annex IV.


Incidence of *Toxoplasma Gondii* and Relationship with Some Inflammatory Factors in Babylon Province

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²Ph.D, Professor, College of Science for Women, University of Babylon, Babylon, Iraq.

Abstract

The study included collecting (60)-blood samples from people who were attending the Children’s Maternity Hospital in Babylon Province, then the Latex test was used to diagnose Toxoplasmosis. The results of the study showed that the percentage of infection with toxoplasmosis in Babylon Province was (11.66%), the infection (IgG) for females was (9.61) out of (52)-samples, and the injury (IgM) for females was (3.84) out of (52) samples, whereas the infection was for males in relation to:

IgG (0%) out of (8 samples) for the total percentage of IgG-against is (8.3%)

IgM (0%) out of (8 samples) and the total percentage of IgM-antibody is (3.3%).

Upon detection of (CRP)-proteins, the study included collecting (60) blood samples for people who were referred to the Children’s Maternity Hospital in Babylon Governorate, and then the (CRP)-test and its relationship to toxoplasmosis were used. The study results showed that the total percentage of active phase proteins for people with toxoplasmosis in Babylon Province, it reached 50% ((the percentage was in females by (53.84%) while the percentage in males was 25%)). The current study showed that the percentage of sensitivity of (IgG, IgM)-antibodies is (8.3%, 3.33%) respectively while the percentage of specificity of (IgG) and (IgM)-opposites was (91.66%, 96.6%) respectively.

Objectives of The Study: Because of the recent spread of toxoplasmosis infection and the increase in miscarriage rates among pregnant women, the present study aimed to determine the infection of this parasite in Babylon Governorate between males and females alike, as well as to know the prevalence of toxoplasmosis and its relationship to the active phase proteins CRP

Keywords: Toxoplasmosis, IgG, IgM, Toxoplasma, gondii, AIDS, CRP, spread, pregnant, female.

Introduction

Toxoplasmosis is an infection caused by a single-celled parasitic organism called a pimping or *Toxoplasma gondii*, which is transmitted to humans through meat that has not been adequately cooked, or through direct contact with animals (especially cats)¹. Usually its symptoms and signs are mild, but in people who suffer from (AIDS) it may lead to severe infection in the brain, while in fetuses that have suffered injuries in early stages of pregnancy may lead to blindness or underdevelopment².

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Gondii curvature (*Toxoplasma Gondii*) is a unicellular parasite that spends most of its life in the carrier of the cat family, especially domestic cats. One of these cat-carrier parasites can transmit huge amounts of it daily, through feces and secretions, which may come in contact or contact with the human being. In addition, toxoplasmosis can travel between all pets that live in one place of residence. The curvaceous parasitic parasite (or *gondii*) parasite is transmitted to the human body by mouth, when eating. A person who is not keen on washing his hands well after touching pet supplies, exposes himself, to a large extent, to this infection. Another additional possibility of contracting toxoplasmosis is eating meat that has not been properly cooked. Because the condylomed parasitoid (*Gondii*) multiplies in the digestive system, it can spread throughout all body tissues, including the brain (3) and blood circulation. The main organs targeted by the parasitic condyloma (*gondii*) are: skeletal muscles, heart muscle, eyes, lymph nodes and lungs. As for patients who suffer from a weak immune system and suffer from diseases, such (AIDS), cancer, or who are being treated with drugs that suppress the immune system, they are more susceptible to infection with toxoplasmosis, in its effective and dangerous form, as a result of exposure to the (*Toxoplasma gondii*) parasite again, or due to viruses in Dormant (dormant) has been developed since a previous infection. This situation is especially dangerous for AIDS (4) patients who, if they have a dormant virus (in the dormant phase) and not treated as it should, could cause severe and severe infections of the brain (encephalitis) that could eventually lead to seizures, convulsions and neurological complications (5).

**Literature Review**

The Toxoplasma gondii parasite is known to cause Toxoplasmosis. It was discovered for the first time before the knowledge that it was a disease in humans was by Laveran in (1900), as explained, but the most important and detailed discovery occurred in the year (1908) by Nicolle and Manceaux confirmed its name in the year (1909) and that During their studies on the northern African biting, Jerusalem, called *Cteriodactylus gondii*, in the Pasteur Laboratories in Tunis, who gave the currently known name to this parasite, the parasite was described in great detail. Around the same time and elsewhere, the scientist Splender described the parasite in a laboratory bunny in São Paulo, Brazil. The first discovery of this disease in an adult human was by the researcher Darling in the year (2) (1908) in Panama, but the first case of toxoplasmosis in an infectious fetus Toxoplasmosis was recorded in humans in (1923), when
he was suffering from a deformed head Hydrocephalus and a small size of the eyes micro–phthalmia. In the late(1930s) and early forties, toxoplasmosis was considered an important infectious disease that affects humans, while Dubey and Beattie stated that the first offensive case of toxoplasmosis in cats was recorded in(1942), when the parasite was identified in the feces of cats after contact with mice infected with the Toxoplasma gondii parasite. The infection occurs through the formation of egg sacs inside the intestines of cats, and from it the role of cats in the transmission and spread of the disease was emphasized(3). In Iraq, Toxoplasma gondii was recorded for the first time by Machattie in(1939) when he observed the parasite in the spleen and lung swabs of a loose dog in Baghdad conducted an epidemiological study in Baghdad to determine the rate of infection with toxoplasmosis in women suffering from Miscarriages, the number of serum wave cases reached (128), i.e.34.7%, for (369) serum mammograms for women suffering from miscarriages through the use of the (IFAT) selection, the yeast linkage for immune absorption (ELISA) and the use of the (LAT-latex) test. Classification of The Parasite:

The final classification of the parasite, as noted(3) by researchers:

**Kingdom** : Protista

**Phylum** : Apicomplexa

**Class** : Sporozoa

**Sub class** : Coccidia

**Order** : Eucoccidia

**Sub order** : Eimeria

**Family** : Sarcocystidae

**Sub family** : Toxoplasmatinae

**Genus** : Toxoplasma

**Species** : gondii

Parasite Forms:

The parasite exists in three stages:

**Tachyzoites:** The name of the active phase with this name is due to the rapid reproduction of the parasite asexually by way of internal budding in the gaps between the cellular tissues of the different host body, This phase is characterized by its crescent shape with a pointed front end and a circular back ground with dimensions of (2-4) micrometers in width (4-8) micrometers in length, and this phase is observed in the acute stages of infection as well as in the chronic stages during re-infection. The parasite is surrounded by an outer envelope composed of an inner and outer membrane, a central nucleus, as well as mitochondria, the collagenous system and ribosomes. This phase is found in different body fluids for the middle and final host such as cerebrospinal fluid, peritoneal fluid and fetal fluid. It is also found in urine, milk, eye secretions, and mucous secretions of the host(1).

**Slowly proliferating shunts and histocysts:** Bradyzoites and Tissue cysts, Tissue cysts form inside the host’s cells to grow and remain in the cytoplasm of its cells with the continuation of the division of the slow-reproducing stages of Bradyzoites inside the cyst. The slow-split phase is similar in appearance to the fast-split phase except for the nucleus of this slow-phase, as it lies near the posterior end of the accurate and surrounds these phases. These phases are also called tissue cysts and that these bags vary in size, ranging in sizes between (100-200) micrometers depending on the numbers of slow-multiplying phases contained in a view that may reach (3000) organisms(1).

These cysts are commonly found in almost all organs during chronic authenticity and have spherical or semi-spherical shapes in the brain (central nervous system) and are compatible with the shape of muscles in the heart and skeletal muscles as well as in the liver, lung, and Czech. Activation cysts activate the ablation of the previous episode, so that the cysts exit the preceding proliferative episode. As well as they contain the red granules that take a red dye(1) when applying pyrodecics-Acid Schiff PAS(2).

**Oocysts-Unsporulated**

They are spherical or semi-spherical in shape with a diameter of (10x12) microns. The bag wall consists of two transparent layers and no polar granules. The sporont often fills the egg bag and sporulation occurs outside the final host body within (1-5) days of its release depending
on moisture and heat. Whereas, the followed bags are semi-spherical to spindle-shaped with a diameter of (11-13) micron. At sporulation, each spore is divided into two splendid sporoblasts, which elongate to form a sporocyst inside each of them creating four spore cells. Egg sacs are the outcome of the sexual process that occurs in the intestine of the final host only \(^2\).

Unsporulated cysts are spherical or nearly spherical in shape and have a diameter of (12x10) micrometers, and the cyst wall consists of two transparent layers and no polar granules, and the two transparent layers form a durable double layer membrane. These cysts form in the epithelial cells of the final host intestine (cats). Unpaired cysts with feces are excreted to the outer circumference and those cysts may grow inside the cat’s intestine to be the active phase. To two sporoblasts that elongate to form the sporocyst, within each of them, four sporozoites of (8×2) microns arise. Egg bags are the outcome of the sexual process that occurs for La Asal, The ovum bags are characterized by their resistance to harsh brown conditions for a long period of up to 18 months in humid \(^2\) environments. It was also noticed that they are not affected by most disinfectants, but they are quickly affected by drought and high temperatures \(^2\). Some insects such as flies, ants, and cockroaches, as well as air currents and rain, play a major role in contamination of human and animal foods. They also work to transfer these ovarian bags from one place to another. It was also found that only ten bags are sufficient to cause infection in humans, while the presence of (100 egg) bags is required for the events of infection \(^2\).

**Life Cycle:** Knowing the parasite’s life cycle is important for controlling the parasite and giving treatment to the patient as well as controlling its vectors and limiting its spread. Although the parasite was described early, knowing the full life cycle was not known until (1970) when Frenkel \(^2\) and his group were the first to assume the life cycle that we know is present for this parasite. Figure(1) shows the existence of two life cycles that include the sexual cycle or the enteroepithelial cycle and it occurs in cats only and the sexual cycle or the extra-intestinal cycle and occurs in cats and intermediate hosts that include many numbers of animals, including rodents, birds and all mammals, including humans \(^3\).

**Sexual Cycle:** Most cats become infected after eating a bird or mouse (infected with a container of tissue cysts) or by eating food or water contaminated with followed (mature) egg sacs excreted in advance with the feces of other sucking cats, so the wall of these cysts will dissolve by proteolytic enzymes in the stomach and intestines and thus slowly proliferating spores and sporozoites are released in the gastrointestinal tract, which penetrate into the epithelial cells of the small intestine and then suffer from asexual reproductive divisions through endodyogeny and schizogeny, resulting in Merozoites, and after several generations, some moles develop by the process of forming large garretogametes, producing large gametes, and female gametes Schoondermark-van de\(^{3}\) in 1995.

After (3-10) days of infection, the segments spread along the small intestine, but they are more in the ileum, and these gametes unite to form a zygote or called a fertilized egg that becomes within a solid wall of development \(^4\). The ungrown egg sac will be thrown with the feces of infected cats for different times between (207) days of infection, as the egg sacs continue to be thrown until about the twentieth day, and the number of egg sacs reaches (100,000 eggs/g) of feces, and these cysts are strong and hard, not infectious, and it is possible to be kept in an outdoor environment for several months. Significantly resistant to dehydration, freezing and disinfectants, but may not be able to survive at (37 °C) for ten minutes. As for the dormant period, which is the period that precedes the laying of the egg sacs, it varies depending on the source of the infection, as it ranges between (3-5) days \(^4\) when the infection \(^3\) is by the tissue cyst, but when the infection occurs with rapidly reproducing vines, it is between (5-10) days and the egg sacs develop. If suitable environmental conditions are available to it, such as oxygen, suitable temperature and humidity, as these cysts swell and reach maturity within (1-3) days, as during this period the vascular vesicles are formed inside the egg sac by a process called sporogony and each sac contains Two spore sacks, each containing four sporophytes, are a source of new infestation \(^3\).

**Asexual Cycle**

The sexual cycle begins in humans and the rest of the other intermediate hosts, as well as cats as an
The intermediate and final host is compulsory at the same time, when humans and other hosts eat the tissue bags found in the meat of infested animals that are not cooked well or egg bags that contaminate water and crops or when in contact with contaminated soil\(^4\). The sporophyte is released from the egg sacs and the slow-growing zygotes from the tissue sacs, and the intestine starts to turn into the rapidly proliferating venules that spread through the blood and lymph to the vital organs and tissues as the first reach of the mesenteric lymph nodes, followed by the liver and the rest of the other tissues, these organisms reproduce within cells by endogenous evolution, as their number reaches \((6-18)\) of rapidly reproducing venules, and as a result, the host cells infected with the parasite are destroyed (causing necrosis and inflammation)\(^5\). After about three weeks of infection, the presence of the parasite is reduced, and that humoral immunity develops. Tissue cysts begin to appear and locate in the nervous, muscle, and cardiac tissues, the diaphragm and the rest of the organs, and these cysts can remain throughout the life of the host, and when the body’s immunity decreases, as in the case of pregnancy or infection with (AIDS), for example, or in people who are immunosuppressed as a result of giving them immunosuppressive drugs. In the case of organ transplantation, these cysts explode, and the slow-multiplication phase that is the beginning of a new asexual\(^5\) cycle is released:

![Figure (2): The Parasite Life Cycle](image)

**Epidemiology**

After toxoplasmosis is one of the most common diseases worldwide, as a study showed that nearly a third of the world’s population is infected with this disease. It has become known that there is a close relationship between chronic toxoplasmosis and the ages of affected persons, as the rates of cationic seroposity appear as the age of those affected\(^6\) increases. Toxoplasmosis is one of the common diseases that are not clinically distinguished, as this disease is widespread (5-95%) in human societies, and it is also an important disease that causes miscarriage, recurrent miscarriage, premature birth as well as malformations. Studies have shown that infections are more common in hot areas than in cold
regions or mountainous regions, and that the variation in the prevalence rate of infection between geographical areas and between population groups within one area may be due to the difference in non-injury. In France\(^{(6)}\), a study conducted on both sexes, it was found that the high prevalence of infection, which amounted to (85%), was related to the population’s preference for eating raw meat, which is the highest rate recorded in Europe, in Central America and in the United States. The incidence increases with the age of the injured to increase by an average of 0.5% to 1% per year of age & Krick. Although clinical toxoplasmosis usually affects only scattered individuals, but small epidemics may occur from time to time. An example of the same occurrence of toxoplasmosis among students of American medical colleges because these students used to eat undercooked pork from the college restaurants as well as what happened from another epidemic in one of the Canadian cities where it was found to be the result of contamination of the source Major Drinking Water for Egg Bags\(^{(7)}\).

In Turkey found that the percentage of the eight positive pregnant women examined was(61.3%) \(^{(7)}\), and in another serological study in Turkey, the percentage of positive tests was(36.4%) of the people. Suspected toxoplasmosis. In a study to investigate antibodies in the serum of aborted women in Turkey, it found an(63.06%) immunoglobulin(g:A) in their serum. While in Iran, there was a variation in the incidence rate, as it reached in its north (70%), while in its south it reached 12%. But in some neighboring countries, including the Kingdom of Saudi Arabia, a study conducted among residents of the Eastern Province in Saudi Arabia showed that the incidence of toxoplasmosis\(^{(9)}\) (25%). Many local studies also indicated an increase in the incidence of toxoplasmosis in Iraq. In Baghdad, recorded an estimated incidence of (34.7%) in aborted women. In the year 2000, Al-Sammani recorded in Mosul an infection rate (39.33%) using the Latex test\(^{(10)}\) and(45.33%) using the indirect telegraphy test. The highest infection rates were recorded among women. Among the women under study, aborted survivors showed the highest incidence of latex grief test. (82.6%) and Daoud and his group (2009) also showed that the incidence of this parasite reached 85% in a study conducted on the group of women in Al-Diwaniyah Governorate.

Al-Nasiri and Daoud also clarified that the incidence was(42% and 41%) among women who had abortions and women who delivered normally, respectively. The study also showed that the percentage of congenital anomalies reached (7%) in children born to mothers with toxoplasmosis\(^{(11)}\).

In Baghdad governorate, a study was conducted to find out the prevalence of infection among males and females, as(2012), Al.khushali found that the infection rate was(40%) and in males and females, respectively(46%).

**Methods of Spread of Toxoplasmosis Infection:**

There are many ways of transmitting the infection, which increases its spread in most of the developed and poor countries of the world alike the methods of transmission of infection to humans can be summarized according to their importance as follows\(^{(11)}\):

1. **Contaminated Food:** This mechanism is one of the most important sources of protection, as it is possible for a person to acquire infection in one of the following methods: eat undercooked and colored meats, especially sheep and pigs, using contaminated food tools such as knives and cooking utensils, eat foods contaminated with uncooked and contaminated meat, eating infected sheep without heat treatment, eating fruits and vegetables contaminated with the excrement of infected animals or grown in soil contaminated with the parasite that causes the disease\(^{(12)}\).

2. **From Animals to Humans:** After human exposure to pets, especially cats, is one of the most common causes of parasite transmission, as the appropriate environment is prepared for its living and reproduction, and then its transmission to humans, and the person becomes ill while cleaning places where colored cats stay or comes into contact with contaminated cleaning equipment, and infection can also occur as a result of contact with contaminated soil when cleaning the garden. The usual presence of cats in it, also showed that a pig or a large sheep may become an important epidemic source at any time, and certainly beef is one of the strong sources of infection, as the sanctuary pigs and sheep are the most vulnerable to pollution, even if it is freezing below (14 °C) for several hours will kill most tissue cysts. Domestic cats will remain one of the dire sources of human infection. As for loose cats, they will lead to multiple problems due to the many
diseases that cats are reservoir hosts for their types. The prevalence of evening cats in rural areas compared to urban families led to a higher\(^{13}\) incidence of infection in rural families (54%) compared with urban (27%) \(^{14}\), and that the spread of the disease in animals was attributed to the high excretion of white sacs by cats. The infected ones that lead to the pollution of the surrounding environment, as pastures, water sources and fodder are contaminated, so the sheep seen in areas without cats do not become infected with toxoplasmosis, while those grazed in the same surroundings and in the presence of cats, the rate of infection with this disease was high and reached (12) in sheep and goats, it was observed that infection occurred due to exposure to stored feed contaminated with cat feces\(^{16}\). Although domestic cats are subject to good supervision and protection by kittens, whether in their food or their livelihood, they may not be free of taking infection indirectly from their environment, and it is possible to lay eggs for several days after injury. That these possibilities are a precursor to a pregnant woman to avoid direct and indirect contact with the necessity to stay away from her locations during at least the period of pregnancy\(^{15}\).

3. **Congenital Transport:** The parasite is transmitted from the infected mother to the fetus, causing congenital toxoplasmosis, when the mother acquires the infection during pregnancy, but in the case of a woman’s infection before pregnancy, the transmission of the parasite to the fetus through the placenta is less likely except in the case of immunosuppressed women. This method is considered one of the most dangerous methods of infection with this disease, especially in the first months of pregnancy, as the disease is transmitted to the fetus through the mother’s sucking placenta for the first time during pregnancy, and the rate of disease transmission from the infected mother to her fetus by the placenta method is (45%) but (60%) of the cases of infection. The newborn has no symptoms, while miscarriage occurs in (9%) of cases, such as abnormalities of the nervous system, eyes and enlarged head\(^{16,17}\).

4. **From Human to Another:** The disease is transmitted from one person to another in very rare cases by organ transplantation, blood transfusion from an infected person to a healthy one, or laboratory personnel coming into contact with contaminated blood or accidentally acupuncture. These cases are by means of preventive treatment until the disappearance of antibodies to the parasite is confirmed. Toxoplasmosis is treated by taking special drugs in doses according to the sex of the infected person. A growth-stopping effect and an influencing activity on the Toxoplasma gondii parasite encouraged its use in the treatment of toxoplasmosis., Therefore, it is considered one of the drugs of choice and common in the treatment of toxoplasmosis in pregnancy.

   In the event that the fetus is infected with the disease, it is necessary for the mother to take appropriate antibiotics such as Pyrimethamine or Sulfadiazine at low doses\(^{78, 79}\) of (25-50) mg per day for a full month, and (10mg) of folic acid should be taken daily\(^{18}\).

**Experiments and Methods:**

**Study Location:** The study was conducted in the Parasitology Laboratory in the Department of Life Sciences of the College of Sciences for Women, University of Babylon.

**Sample Collection:** 30 blood samples were collected from reviewers of both sexes for the laboratories of Maternity Hospital for Children. Use sterile plastic tubes to collect the samples (blood samples), then they are numbered and some private information is recorded for each references. The blood was separated by using a centrifuge at 3000 rpm for five minutes. Keep the work in numbered plastic tubes at a temperature of 25 °C until the examination is done.

**Serological Examination to Diagnose Toxoplasmosis:**

**Latex Agglutination Test CLAT:** In this test, several commercial tests produced by a company called (S.A.SPAIN) and (SPINREA) were used to detect the presence of specialized antibodies against the parasite Toxoplasma gondii ,and the kit consists of the following components\(^{19}\):

1. **Latex Reagent** is a suspension of latex granules made from Polystyrene fertilizer and covered with antigen dissolving the parasite in a circulating saline solution that contains a preservative Sodium azid with a concentration(0.95%), that the latex granules enables to notice the correlation resulting from the interaction The antibody and the antigen with the naked eye or under
the microscope with a magnification power (10), as the clumping appears in the case of the presence of the serum antibodies to be examined.

2. **Positive Control Reagent:** It is a human serum that energizes the specialized antibodies against the anti-Toxoplasma parasite and contains (0.95%) of sodium azide.

3. **Negative Control Reagent:** It is animal serum free of specialized antibodies against the parasite, it contains (0.95%) of Sodium Azide. The kit should be kept in the refrigerator (19), in degree 4” until use.

**The Methods (19):**

1. (10 μl) of the sample was taken
2. This sample was mixed with latex reagent (5μl).
3. Then put Latex-cell slice
4. Then wait for (10) minutes until you notice the presence of the line or the mark by which the color changes to a red color or a red line

**Note:**

A . The line indicating (T1-Toxo IgG) indicates that the test is positive for this antibody.

B . The line indicating (T2-Toxo Tgm) indicates positive for this antibody.

C. If neither of the above statements appears, this indicates that the sample is negative

D. But if it appears to both of them, it indicates that both of the two opposites (Igm, IgG) are positive.

---

**Figure(3): Latex Cassett agglutination test for IgG and IgM**
Methods for CRP

C-Reactive Protein: The concentration of the activity proteins is measured by the latex method. Latex, if detected by means of the Slide agglutination test, if the qualitative estimation and quantification of the quantitative ratio are used to measure the active phase protein and that the positive result is agglutination as a result of the removal of human antibodies working with the active phase protein with the active phase protein present in the patient’s serum sample(20).

Qualitative Method for Measuring of Protein Concentrations:

1. The reagent components and samples are left at room temperature.

2. A drop of serum was placed in the designated chamber on the test strip.


4. The two drops (serum+ reagent) were mixed well and then we spread well the circuit on the test strip(87).

5. The slide was moved in a circular motion for two minutes, as the result was shown in clumpy form.

Results and Discussion

The following table shows that the total number examined is 30 patients. If the percentage of infection was (IgG and IgM), as well as the total number examined and the number infected with toxoplasmosis and not infected:

The results in Table.(1), showed that the percentage of positive cases that appeared when using (C-Reactive Protein) for infected and non-infected cases of toxoplasmosis is (50%) of the number of samples of (60) samples., Between the percentage of infection with toxoplasmosis and its relationship to the sex of the patient in (IgM), it was noticed that the infection was also concentrated in females by (3.84%), while it was also Table No.(1) act (0%) for males.

Table.(1):Resulting Data of Samples

<table>
<thead>
<tr>
<th>Total Examined Samples</th>
<th>Not. Infected</th>
<th>Infected</th>
<th>Infection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG : 60</td>
<td>5</td>
<td>55</td>
<td>8.3 %</td>
</tr>
<tr>
<td>IgM : 60</td>
<td>2</td>
<td>58</td>
<td>3.3 %</td>
</tr>
<tr>
<td>CRP : 60</td>
<td>7</td>
<td>53</td>
<td>11.66 %</td>
</tr>
</tbody>
</table>

Number of positive cases when detected by (C-Reactive Protein) for acute and non-toxoplasmosis cases.

<table>
<thead>
<tr>
<th>Total Examined Samples</th>
<th>Positive Cases</th>
<th>Negative Cases</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>30</td>
<td>30</td>
<td>50 %</td>
</tr>
</tbody>
</table>

Percentage of toxoplasmosis and its relationship to the patient’s sex relative to(IgM)

<table>
<thead>
<tr>
<th>patient’s sex of IgM</th>
<th>Positive Cases</th>
<th>Negative Cases</th>
<th>Infection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>2</td>
<td>50</td>
<td>3.84 %</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0 %</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>8</td>
<td>3.3 %</td>
</tr>
</tbody>
</table>
Which indicated that the percentage of infection with toxoplasmosis and its relationship to the patient’s psychological sex as for Table(2) for males. The (IgG) antibody, it was noted that the infection was concentrated in females by (9.61%), while it was (0%). The results showed Table.(2) that the percentage of infection with Saint Mary’s disease infected and not infected) and its relationship to the sex of the infected patient. And non-infected) when measuring (C.reactive protein), females had a greater concentration of this protein by (53.84%), while males were (25%), while the total number of infection and its relationship to the concentration of acute phase proteins was (50%).

Table.(2): The percentage of toxoplasmosis infection for samples

<table>
<thead>
<tr>
<th>patient’s sex of IgG</th>
<th>Examined Samples</th>
<th>Positive Cases</th>
<th>Negative Cases</th>
<th>Infection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Females</td>
<td>52</td>
<td>5</td>
<td>47</td>
<td>9.61</td>
</tr>
<tr>
<td>Total .No</td>
<td>60</td>
<td>5</td>
<td>55</td>
<td>8.3 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>patient’s sex - measuring (C.reactive protein)</th>
<th>Examined No.</th>
<th>Positive Cases</th>
<th>Negative Cases</th>
<th>Percentage of Toxoplasmosis %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>52</td>
<td>28</td>
<td>24</td>
<td>53.84 %</td>
</tr>
<tr>
<td>Males</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>25 %</td>
</tr>
<tr>
<td>Total .No</td>
<td>60</td>
<td>30</td>
<td>30</td>
<td>50 %</td>
</tr>
</tbody>
</table>

Calculation of Average for Sensitivity and Specificity Rates

The percentages of sensitivity and specificity were extracted according to the rates shown below:

Sensitivity = number of Real positive cases / number of Real positive cases + number of false negative cases x 100%

IgG = 5/5 + 55 X 100% = 5/60 = 8.3%
IgM = 2/60 X 100% = 3.33%

Specificity = number of Real Negative cases / number of Real Negative cases + number of false Positive cases x 100%

IgG = 55/5 + 55 X 100% = 55/60 = 91.66%
IgM = 58/60 X 100% = 96.66%

Toxoplasmosis is an epidemic disease of global prevalence, especially in tropical and subtropical regions. Also, toxoplasmosis is spread in the environments in which it is spread and other animals that are domestic with humans. The unhealthy environmental conditions in
these areas are among the most important factors for the spread of this disease, in addition to the loss of personal hygiene factors, mixing with domestic animals and cats, and eating meat of animals that are not well cooked are among the most important factors in the spread of the disease, especially in Iraq. It was found from the current study that the ratio of (IgG) antibody in the current study and (IgM) antibody is consistent with the study and not consistent with the study. Perhaps these ratios are due to the difference in the size of the sample taken and the method of work used and it appears from the results. Compared with (IgM), this confirms that most of the infections are acute, given that the (IgG) antibody is associated with chronic infections, and (gM A) is associated with acute injuries. The results showed that the percentage of (IgG) antibodies to (IgM) was the highest in females in disease resistance respectively compared to males (5%) for both antibodies and this is consistent with the study and not consistent with the study of both the studies, this is due to the size of the sample taken in the current study, as the number of samples examined was out of (30 samples) from different people, so this ratio appeared as we conclude that females are less immune than males in fighting germs and diseases. Finally, the current study showed that acute (CRP)-proteins and their association with toxoplasmosis appeared high with (IgG) antibodies and then (IgM). It is known that these proteins are immune proteins that are increased with chronic infections, which are mainly associated with (IgG) antibodies because they are associated with chronic infections. This is in agreement with the study and not in agreement with studies(21,25).

Conflict of Interest: There is no any Conflict of Interest

Ethical Clearance: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

Source of Funding: Self funding.

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Phylogenetic Analysis of TTV (Torque Teno Virus) in Iraqi Woman Suffering of Failure Kidney Disease

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1Assistant Professor, College of Nursing, 2Tutor, Department of Pharmacy, Al-Rasheed University College

Abstract

There are still very few studies of the Torque Teno virus infection rate between hemodialysis patients in Iraq. Thus, the aim of this study was to assess the frequency of TTV viremia in hemodialysis Iraqi patients. This study has carried out in Baghdad city from October 2019 to February 2020 and included 150 patients with kidney failure disease undergoing hemodialysis and 50 control subjects. Blood samples have been obtained for serological TTV detection. The result shows PCR has been used to detect TTV, isolates have been identified through N22 region sequencing and phylogenetic analysis has also been performed. Of the 150 patients, 2 (1 male and 1 female) were TTV positive. The results show in (isolate 14) substitution four Transversion A>C, C>G, A>T, and T>A three Transition T>C, A>G, and T>C of Specific region within TTV(N-22) and showed 94% identified with a standard in Gene Bank while having 99% identified with a standard in Gene Bank with (isolate 17) substitution five Transversion A>T, A>C, T>A, A>T and C>G three Transition A>G, A>G and T>C. The phylogenetic analysis showed that identical among themselves and the world with 100% compatibility values.

Key words: kidney failure disease, Torque Teno virus (TTV), PCR- Sequencing.

Introduction

The Torque Teno virus (TTV) was initially isolated from a post-transfusional non-A-G hepatitis patient in Japan (1). The virus is an omnipresent negative stranded DNA virus which has been listed as an Anelloviridae family member (2). Though considered a hepatotropic virus, with different transmission modes (3). TTV is classified as into seven genogroups including many genotypes according to sequence analysis (4), of which genotype 1 is the most prevalent. The virus is spread globally according to geographic location with variable seropositivity levels (5,6). TTV usually infects patients exposed to blood transfusion as patients with thalassemia and haemodialysis, also intravenous users of the drug (7). The virus has been rendered pathogenic and is also considered a virus flora, but its significance stems from its capability to demonstrate the combined capacity from the innate and acquired immunity (8). Despite the innumerable utility of haemodialysis in chronic renal failure patients, it has been attributed as a high danger setting for blood-borne infections, that may be appliances, surfaces and staff of various origins in haemodialysis units (9). Besides HCV and HBV, TTV are among the frequently transmitted viruses in patients with haemodialysis, the frequency of which varies greatly contingent on virological, demographic and clinical factors (10). Thus the aim of this study were to assess the frequency of TTV viremia in hemodialysis Iraqi patients.

Materials and Methods

This study has carried out in Baghdad city from October 2019 to February 2020 and included 150 patients with kidney failure disease undergoing hemodialysis and 50 control subjects who were apparently healthy. Five ml of whole blood was collected from each subject. Blood sample was allowed to clot naturally then centrifuged at (3000 rpm) for 5 minutes and serum was separated from packed RBCs. Serum was divided into 2 parts in sterile Eppendorf tubes which then stored in deep freeze at -20°C, one part was used for DNA extraction from TTV, and the second part for detection of TTV by ELISA.
Detection of TorqueTeno virus by ELISA

Detection of TTV were done firstly by ELISA (Cat. No: MBS9313728, MyBioSource, USA).

Amplification of TTV by using PCR

Specific region within TTV(N-22) amplified by using PCR with forward primer 5'-GTC AAG GGGCAA TTC GGG CWC-3’ and reverse primer 5'-GTC TGG CCC CAC TCA CTT TCG-3’. The program of PCR as seen in table (1), PCR products were verified in 1.5% agarose gel.

Table (1): PCR program that was applied in the thermocycler devices.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation 1</td>
<td>95°C</td>
<td>5min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation 2</td>
<td>95°C</td>
<td>20sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>60°C</td>
<td>20sec</td>
<td>35</td>
</tr>
<tr>
<td>Extension 1</td>
<td>72°C</td>
<td>20sec</td>
<td></td>
</tr>
<tr>
<td>Extension 2</td>
<td>72°C</td>
<td>5min</td>
<td>1</td>
</tr>
</tbody>
</table>

Phylogenetic analysis

Two TTV isolates from this samples were utilized for phylogenetic analysis to establish the genetic relationship between Iraqi human TTV strains and the NCBI data base strains. Using the MEGA6 software, the nucleotide sequences were aligned based on the amino acid sequences to prevent nonsense from being introduced.

Results

The distribution of the samples according to gender showed insignificant distribution ($\chi^2=5.9017 , P>0.206$), the negative samples showed 63 females and 85 males while the two positive samples were divided equally into one male sample and one female sample. The control samples were 23 males and 27 females.

Table (2): samples distribution according to gender.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>TTV negative</td>
<td>63</td>
<td>85</td>
</tr>
<tr>
<td>TTV positive</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chi-square</td>
<td>5.9017</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.206</td>
<td></td>
</tr>
</tbody>
</table>

The results of ELISA test showed 21 samples were positive (out of the total 150 samples). ELISA test has low Specificity of results (31.58%). The percentage of $TTV$ positivity were different after the PCR test were done as shown in table (3).
Table (3): Comparison between ELISA and PCR techniques.

<table>
<thead>
<tr>
<th>Specificity of ELISA %</th>
<th>percentage</th>
<th>positive samples</th>
<th>Tests</th>
<th>Total collected samples for patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.58%</td>
<td>14%</td>
<td>21</td>
<td>ELISA</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>1.30%</td>
<td>2</td>
<td>PCR</td>
<td></td>
</tr>
</tbody>
</table>

The results of aligning the sequences results of TTV are illustrated in table (4) which showing the variation of each sample. Each sample showed different variations. The first sample showed 7 variations which are; at nucleotide 112 (TTT > TTA), at nucleotide 178 (AAC > ACC) missense, a nonsense at nucleotide 182 (AAT > AAC), at nucleotide 194 (GGC > GGG), at nucleotide 196 (TAC > TCC), at nucleotide 200 (GTA > GTG) and at nucleotide 206 (TAT > TAC). The other sample showed 8 variations at which are; at nucleotide 50 (AAC > GAC), at nucleotide 127 (TAC > TCC), at nucleotide 123 (AAC > GAC), at nucleotide 176 (AAC > ACC), at nucleotide 179 (ATC > ACC), at nucleotide 198 (TTT > TTA), at nucleotide 201 (TAC > TTC) and at nucleotide 219 (GGC > GGG). Those variations resulted after aligning the sequence of our isolates to the reference sequence (ID: AF123933.1) which showed the highest similarity to our resulted sequence NCBI.

The phylogenetic tree diagrammatic by Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 the phylogenetic trees of these species are shown in Figure (1). These alignments appeared the TTV between Iraq and others in the world by Specific region within TTV (N-22) for translating specific region. Hierarchical cluster analysis determine the following clusters including TTV Iraqi isolate (14,17) the identical 100 % it is close to Egypt (ID: KY750545) the identical 100 %.

![Figure 1: Neighbor-joining tree from TTV sequence alignment.](image-url)
Discussion

The patients with chronic renal failure suffering from hemodialysis (HD) are at risk of TTV infection (11). Data on TTV infection in peritoneal dialysis patients was very rare; these results indicated the prevalence of TTV in a population with continuous ambulatory peritoneal dialysis is close to that of healthy controls (12). At worldwide distribution, torque teno virus was found to be more widespread in men (13). Spandoleet al (14) reported that the distribution from TTV in males and females did not notice any difference, this agreement with our study. At the other hand, Mohamed et al (15) recorded that TTV-positive patients were 66.7 % and 33.3 % respectively in age groups 20-40 and 40-60 years, 3.7 % TTV-positive DNA were males compared to 6.1 % of females with no noticeable difference between males and females. Al-Hamdaniet al (16) found that 29.2 % from thalassemia patients with predominance of males over females were detected with TTV (64.4 % vs. 35.6 %). Takemoto et al (17) reported that patients with dialysis were mostly males (55 %) with a mean age of 53.8 years. The current study showed the relationship between TTV infection and patients suffering from hemodialysis in a population of kidney failure. The discovery of TTV and its parenteral mode of transmission resulted in the idea of its potential inclusion in a category of viral hepatitis that is not A-G (18). Sequence analysis shows that there is a high degree of genetic variation in the TTV genome and can be categorized into at least six main genotypes (groups 1–6) and multiple subtypes (1a, 1b, 2a and 2b) (19, 20). Direct UTR region amplicon sequencing revealed extensive mix-infection of different TTV strains within the infected person. The divergence among various detecting regions indicated that TV is due to the coexistence of multiple phylogenetic groups (4). In the current study the sera samples from failer kidney disease patients and control were were assayed for the existence from TTV-DNA utilizing PCR and amplifying specific zone of TT genome. To determine the phylogenetic relationship of Iraqi isolated strains among human TTV, sequences from GenBank and isolates from the present study were included for phylogenetic analysis. The findings of the ELISA technique showed that 21 samples were positive (out of a total of 150 samples), has low Specificity specificity (31.58%) as compared with the PCR diagnosis of the Sanger Sequencing technique for Torque teno virus with high results specificity. The polymerase chain reaction diagnostic techniques is rapid, easy, inexpensive protocol becoming the most commonly utilized of all molecular genetics ways for detecting important genes. Irshad’s analysis found that Torque teno virus DNA genotype 1 was the principal genotype of TTV DNA in patients with hemodialysis (21). In contrast, genotype 2 was reported to be more pathogenic than other genotypes (22, 23). Likewise, a study carried out in Turkey to examine the N22 region found that genotypes 1 and 2 were the most common in the different groups investigated and the only one identified in the population of blood donors (24). Torque teno virus has been spread worldwide, with a high prevalence of TTV infection in various populations including liver disease patients, HIV patients, drug users and healthy individuals (25). Irshad et al (18) reported that PCR fragment molecular and phylogenetic analyzes detected that TTV could be divided into many genotypes found worldwide, without any direct connection to the geographic distribution of diseases. This disparity in the prevalence of TTVs in various geographic areas can be explained by the presence of different roads of this virus spreading across different geographic areas. Castro et al (23) represented a molecular epidemiological study examining a large number of Brazilian isolates and reported that TTV was identified among 11.9% of Brazilian blood donors through the study of the TTV genome ORF-1 region close to that found between German and Japanese blood donors.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References


Comparison of Different Diagnostic Techniques for the Identification of Pulmonary Tuberculosis and Using Serum Tnf-A Levels as a Biomarker for Evaluating the Severity of Tuberculosis

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Abstract

Background and Objective: Pulmonary tuberculosis(PTB) is a serious disease that mainly affects the lungs. The aim of this study was a Comparison between conventional, and molecular methods to diagnose M.tuberculosis complex (MTBC) and using serum TNF-α levels as a biomarker for evaluating the severity of the disease. Methodology: The study analyzed 586 sputa collected from suspected pulmonary tuberculosis patients (217 female and 369 male) their ages ranged from 16 to 66 years for mycobacteriological study, while the immuno-study included 85 blood samples that collected from eighty patients and five healthy persons (45 male,40 female) their ages (18-66) years. We tested all sputum samples by AFB direct stain and GX methods, while we cultured sputum restrictively for Patients who have two different outcomes by AFB direct stain and GX methods. TNF-α levels have been measured by sandwich ELISA in patients and healthy persons. Results: Our result showed detection of (135/586) patients infected with M.tuberculosis complex (MTBC) and (1/586) patient infected with non-mycobacterium tuberculosis(NMT) by AFB direct stain and GX methods comparing with culture method as a gold standard. Sensitivity and specificity for AFB direct stain and GX techniques for diagnosis PTB were (15.0% and 100% ) and (95.0% and 66.7% ) respectively. On the other side, TNF-α levels were elevated in inpatient groups compared with healthy persons. The mean of TNF-α levels measured in multidrug resistance tuberculosis (MDR-TB) patients was higher than in other cases. Conclusion: Our findings demonstrate that the sensitivity of the GX technique is higher compared to other techniques, and serum TNF-α levels can be used as a biomarker for evaluating the severity of pulmonary tuberculosis.

Keywords: Pulmonary tuberculosis, MDR-TB, TNF-alpha, GeneXpert MTB\RIF, BACTEC MGIT960.

Introduction

Tuberculosis (TB) remains the single largest infectious disease, a total of 1.5 million people died from TB in 2018 (including 251 000 people with HIV). This disease occurs as a result of infection by a group of bacteria such as M.tuberculosis complex(MTBC) and non-mycobacterium tuberculosis(NMT).1,2

Diagnosis of pulmonary tuberculosis by conventional diagnostic techniques is difficult and required a long time for detection. AFB direct stain technique is still commonly used, especially in developing countries, although it has low sensitivity and normally requires (3,000-5,000) bacilli per ml of sputum to be positive. The culture method is a gold reference that provides a definitive diagnosis of tuberculosis by establishing the viability and identity of the pathogens.3

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GeneXpert MTB\RIF technique developed by Cepheid, USA, that is a fully automated system that can be easily used, and it depends on 81 bp sequences located within the core region of rpoB gene which coded for Beta-subunit of RNA polymerase enzyme, it can detect MTBC and determines whether they are resistant to rifampicin simultaneously in a short time.4

TNF-α is a cytokine that mediates mainly the intercellular communication and plays a major role in the protection against M. tuberculosis and regulates the cellular immune response, especially in the early stage of infection. Also, this cytokine participates in the processes of the host’s defense against pathogens, inflammatory, and reparation of tissue.5

The course of tuberculosis is dependent on the interactions of the M.tuberculosis and the immune system of the host. When person inhalants air droplets containing mycobacteria and arrive at its target(epithelial cells of the lung), innate immunity such as macrophages are rapidly activated and the adaptive immune system such as T-lymphocytes synergistically cooperates for preventing of growth and spreading of the bacteria.6,7

Material and Methods

2.1- Participants groups and Clinical Samples Collection

We conducted a cross-sectional study from September 2019 to February 2020 in the International Center for Chest and Respiratory Disease in the Kurdistan provinces of Iraq ( Erbil, Dohuk, and Sulaymaniya cities). A total of 586 sputum samples collected from suspected PTB patients to identify MTBC. Positive patients with PTB classified into two groups; (114/136) recently diagnosed patients (63 male, 51 female) and (22/136) multidrug resistance M.tuberculosis patients (11 male, 11 female), their ages ranged from 16 to 66 years for mycobacteriology study, while the immunological study was included collect five milliliters(5 ml)of blood from eighty patients from the following groups; (48/80) under-treatment patients(26 male, 22 female), (10/80) patients from recently diagnosed patients (5 male, 5 female), (22/80) patients with MDR-TB (11 male and 11 female), as well as five healthy persons (3 male, 2 female) their ages, ranged from 18 to 25 years.

In this study, we identified MTBC and NMT by AFB direct stain and GX methods comparing with culture method to evaluate the accuracy-test of these techniques. On the other hands, we used TNF-α levels as a biomarker for evaluating severity of PTB.

The following methodology was followed for samples processing ;

2.1.1-AFB direct stain: All specimens were stained by Ziehl-Neelson staining according to manufacturer’s recommended method (Kimadia, Iraq).8

2.1.2-Culture method: We cultured sputum restrictively for the following candidates; Patients with two different outcomes by AFB direct stain and GX techniques (e.g., GX - ve cases but AFB + ve or vice versa) as well as 10 of 46 sputum samples were GX +ve and AFB +ve and 10 of 510 samples were AFB -ve and GX –ve to check the positive and negative of these techniques respectively” Diagram 1”. Only 115 sputum samples cultured according to the laboratory standard procedure for primary isolation.8,9

Diagram 1: Flow work of all diagnostic techniques. GX: GeneXpert, AFB: acid-fast bacilli method.

2.1.3-GeneXpert MTB\RIF technique:

For identifying MTBC and RIF resistance by GX technique, we tested all suspected sputum samples according to method recommended by the manufacturer 10,11,12

2.1.4-Differentiation between MTBC, MTB, and NMT:

We used niacin accumulation, nitrate reduction and ability of MTB to growth on L.J medium contains 500 µg/ml of Para-nitro benzoic acid to differentiate between M.tuberculosis and MTBC (Kits provided from HIMedia [India] for niacin acumination and L.J medium with PNB kits, while nitrate reduction kit provided from COTREZ[USA] )

While, IS6110&ITS method used to differentiate between MTBC and NMT[RealLine Extraction 100 kit provided from BOIRON, Germany] for Extraction DNA, and prime TB/NTM detection kit provided from [GeNet BIO, South Korea] for the amplification) according to method recommended by the manufacturer.13,14,15
2.1.5- **Drugs susceptibility test (DST):** After 4 weeks of incubation on the L.J medium, DST was performed for first-line antitubercular drugs according to the working method recommended by Becton Dickinson’s product and procedure manual (BD Biosciences, MD, USA) by BACTEC MGIT960 and SIRE kit procedure.  

2.1.6- **Measurement of serum TNF-α levels:** 5 ml of blood were collected from 85 participants. We measured serum TNF-α levels for 80 patients comparing with five healthy persons. The quantitative sandwich ELISA method has been used for assessment of TNF-α serum levels, the kit provided from (Al-shkairate establishment, Jordan), and the procedure of kit recommended by the above company has been followed.

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### Statistical analysis

We used IBM SPSS 22.0 to analyze the study data. To compute the statistical difference between all the sensitivities of the diagnostic techniques, we used the Chi-square test at a confidence interval (95%), while the T-test was used to account for the differences between the levels of TNF-α. Also mean±standard deviation (SD) used to represent TNF-α levels. A p-value less than 5% was considered statistically significant. All graphs were generated using Microsoft Excel, 2010. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy test for all diagnostic techniques were calculated based on Baratloo et al.  

### Results

#### 3.1-Isolation and identification of M.tuberculosis complex (MTBC)

Our results showed a diagnosis of 135 (23.0%) patients infected with MTBC and one patient was infected with NMT (0.34%) of 586 suspected PTB patients by AFB direct stain and GX diagnostic methods, and compared these results with culture method (a gold standard) to confirm the diagnosis and calculate sensitivity, specificity, and accuracy test for AFB and GX techniques.  

![Figure 1](image-url)  

**Figure (1): Results of different diagnostic method.**

<table>
<thead>
<tr>
<th>Type of sputum examination</th>
<th>No. of positive isolates n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB + ve \ GX +ve</td>
<td>46(7.8)</td>
</tr>
<tr>
<td>AFB + ve GX –ve</td>
<td>5(0.9)</td>
</tr>
<tr>
<td>AFB –ve \ GX +ve</td>
<td>85(15.3)</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>136(24.0)</td>
</tr>
</tbody>
</table>

p<0.05
**GX= GeneXpert, AFB= Acid-fast stain**

AFB direct stain method was able to identify 51 (8.7%) samples out of 586 sputum samples (46 isolates were GX +ve, while 5 isolates were GX -ve). On the other hand, GX was able to detect (136/586) samples (90 of 535 were –ve AFB [5 of them were false-positive samples] and 46 of 51 samples were + ve AFB). It is worthwhile that the GX method could not diagnose five samples which were positive by AFB direct stain and culture techniques (false negative samples), p<0.05.

“Table 1 and Fig.1”.

The culture method was able to confirm diagnosis (100/115) sputum samples, including false negative samples that didn’t diagnose by the GX method (p<0.05), “Table 2 and diagram 1”. Sensitivity, specificity, PPV, NPV and accuracy test comparing with the culture (gold standard) for all diagnostic techniques showed in the “Table 3”

Table 2. Outcomes by different tuberculosis diagnostic methods

<table>
<thead>
<tr>
<th>Results of GX and AFB techniques</th>
<th>Results of Culture technique</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive result</td>
<td>Negative result</td>
</tr>
<tr>
<td>GX-ve\AFB-ve (445/586) isolates</td>
<td>(0\10)</td>
<td>(10\10)</td>
</tr>
<tr>
<td>GX+ve\AFB-ve (90/586) isolates</td>
<td>(85\90)</td>
<td>(5\90)*</td>
</tr>
<tr>
<td>GX+ve\AFB+ve (46/586) isolates</td>
<td>(10\10)</td>
<td>(0\10)</td>
</tr>
<tr>
<td>GX-ve\AFB+ve (5/586) isolates#</td>
<td>(5\5)</td>
<td>(0\5)</td>
</tr>
<tr>
<td>Total</td>
<td>(100\115)</td>
<td>(15\115)</td>
</tr>
</tbody>
</table>

P-value< 0.05

*False positive of GX. #: False negative of GX. AFB= Acid fast bacilli stain technique, GX= GeneXpert

Table 3. Sensitivity, specificity, PPV, NPV, and accuracy test for all techniques.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>AFB stain technique</th>
<th>GX technique</th>
<th>AFB vs GX</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity(%)</td>
<td>15.0</td>
<td>95.0%</td>
<td>90.2</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Specificity(%)</td>
<td>100</td>
<td>66.7</td>
<td>83.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>PPV(%)</td>
<td>100</td>
<td>95.0</td>
<td>33.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>NPV(%)</td>
<td>15.0</td>
<td>66.7</td>
<td>98.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Accuracy test(%)</td>
<td>26.0</td>
<td>91.3</td>
<td>83.3</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

GX= GeneXpert, AFB= Acid fast stain PPV &NPV= positive & negative predicative value respectively.

3.2-Differentiation tests of MTBC and NMT

Our results showed that (91\100) isolates belonged to MTB, and (9\100) isolates belonged to MTBC other than MTB, while the IS6110&ITS method differentiated (1\5) isolate belonged to NMT, as well as (4\5) isolates were MTBC.
3.3-Drugs susceptibility Test(DST)

To find the accuracy test of the GX technique for detection RIF resistance isolates, we compared between GX and BACTEC MGIT960 techniques, the first technique detected (23\text{\%}) RIF resistant isolates while the second technique identified (22\text{\%}) RIF resistant isolates. The sensitivity and specificity for GX technique compering with BACTEC MGIT960 (gold of standard) were (100\% and 98.5\%) respectively (P < 0.05), “Table 4”. It is worth noting that (1/23) isolate which detected by GX technique was false resistance, this isolate did not detect by BACTEC MGIT960 technique.

Table 4: Comparsion of GeneXpert MTB\RIF with BACTEC MGIT 960 results.

<table>
<thead>
<tr>
<th>GeneXpert results</th>
<th>BACTEC MGIT 960 results</th>
<th>Total n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance n (%)</td>
<td>Resistance n (%)</td>
<td>22(24.2)</td>
</tr>
<tr>
<td></td>
<td>Sensitive n (%)</td>
<td>1(1.1)</td>
</tr>
<tr>
<td></td>
<td>Total n(%)</td>
<td>23(25.3)</td>
</tr>
<tr>
<td>Sensitive n (%)</td>
<td>Resistance n (%)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>Sensitive n (%)</td>
<td>68(74.7)</td>
</tr>
<tr>
<td></td>
<td>Total n(%)</td>
<td>68(74.7)</td>
</tr>
<tr>
<td>Total n(%)</td>
<td></td>
<td>22(24.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69(75.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>91(100)</td>
</tr>
</tbody>
</table>

P-value < 0.05

True Resistance(a) = 22
False Resistance(b) = 1

True sensitive(c) = 0
False sensitive (d) = 68

Analysis values 95\% CI

<table>
<thead>
<tr>
<th>Sensitivity(%)</th>
<th>a/a+c\times100= 100%</th>
<th>(84.56- 100.) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity(%)</td>
<td>d/d+b\times100= 98.5%</td>
<td>(92.19- 99.9) %</td>
</tr>
<tr>
<td>Positive predicative value(%)</td>
<td>a/a+b\times100= 95.6%</td>
<td>(75.87 99.35) %</td>
</tr>
<tr>
<td>Negative predicative value(%)</td>
<td>d/d+c\times100=100%</td>
<td>--------</td>
</tr>
<tr>
<td>Accuracy test(%)</td>
<td>(a/c)/(b/d)= 98.9%</td>
<td>(94.03- 99.97)%</td>
</tr>
</tbody>
</table>

3.4- Measurement of serum TNF-\(\alpha\) levels cytokine.

We measured serum TNF-\(\alpha\) levels cytokine in patients comparing with healthy persons. Our results showed that the serum TNF-\(\alpha\) levels were high in patients comparing with healthy persons. On the other hand, The mean serum TNF-\(\alpha\) levels measured in MDR-TB patients were higher than those of RD and UT patients (P < 0.05) “Table 5”.


Table 5. Serum levels of TNF-α in studied groups

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>The concentration of TNF-α Pg/ml</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Maximum</td>
</tr>
<tr>
<td>MDR</td>
<td>467.7±83.613</td>
<td>485.5</td>
</tr>
<tr>
<td>RD</td>
<td>332.5±48.421</td>
<td>478.9</td>
</tr>
<tr>
<td>UT</td>
<td>46±16.06</td>
<td>199.3</td>
</tr>
<tr>
<td>Control</td>
<td>20.9±4.525</td>
<td>23.9</td>
</tr>
<tr>
<td>Total</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

P-value< 0.05

MDR: multidrug resistance, RD= recently diagnosed, UT: Under-treatment, TNF- α: tumor necrosis-α

Discussion

Diagnostic methods for detection PTB are constantly evolving to find fast and accurate techniques to directly detect MTB. Because both controls of PTB infection and management of patient are dependent on an early and accurate diagnosis of PTB. This study presented important information about the sensitivity of different diagnostic techniques and role of TNF-α cytokine for evaluating the severity of PTB.

Our findings showed that AFB direct stain technique has low sensitivity(15.0%) and high specificity(100%). These results were closely similar to Agrawal et al, who found low sensitivity(22.2%) and high specificity(100%) for AFB method, while Odubanjo & Dada-Adegbola, found that the sensitivity and specificity of AFB direct stain were (74.5% and 71.6%) respectively. This could be attributed to many factors affect the sensitivity of AFB stain as; Number of bacilli present in sputum that requires 3,000-5,000 bacilli per ml of sputum to be positive, prevalence and severity of the disease.

Also, our results showed high sensitivity(95%) and good specificity(66.7%) of GX technique comparing with culture, these results were similar to Bunsow et al, who found high sensitivity (97.1%), and specificity(99.1%) for Pulmonary specimens.

It is worth noting that the results of our study showed that five false positive cases (GX+ve\AFB-ve, and culture –ve), in addition, five false negative cases of GX method, which were positive by AFB direct stain and culture methods(one of them were NMT). The reason for these is attributed the GX can detect the DNA even for dead and fragmented bacteria which is considered as a major disadvantage of this technique, therefore, GX technique cannot be used for follow-up of patients after the treatment, as well as maybe due to presence of PCR inhibitors in the sputum, although GX included a positive control for detection the inhibitors, or maybe due to presence NMT in sputum.

On the other side, out of 91 MTBC isolates diagnosed by GX technique, this machine detected 23 RIF resistance isolates, while MGIT960 machine detected 22 RIF resistance isolates, Sensitivity and Specificity of GX machine were 100% and 98.5%, respectively. Our findings were similar to Laskar et al, showed that sensitivity and specificity of GX were 87.64% and 75% respectively. Also, we found one isolate was resistance for RIF that was detected by the GX technique, while it was susceptible to RIF by BACTEC MGIT960. This case may be attributed to the presence of a silent mutation within the rpoB gene core region.

In the immunological part of our study, we found that serum TNF-α levels were high in patients comparing with control and the elevation was higher in MDR-TB than other groups(UT and RD).
These results were closely similar to Shameem et al., 27 who found that serum TNF-α levels were high in MDR-TB and RD patients, while the levels significantly decreased in UT group comparing with healthy persons. Also, they found high levels of TNF-α in MDR patients comparing with recently diagnosed patients who were drugs susceptible.

Also, Andrade Júnior et al., 28 found that levels of TNF-α were high with significant differences between tuberculosis and control groups and There was a positive correlation between patients’ clinical severity and the serum TNF-alpha levels; but, they found a slight increase in TNF-α levels of patients with MDR-TB. The reason for the complete incompatibility between the results of the two studies may be due to the small number of subjects in their study compared with the number of subjects in our study(80 patients), as their study included 24 patients, three of them had MDR-TB only.

**Conclusion**

Our findings demonstrate that the sensitivity of the GX technique is higher compared to other techniques, especially in negative direct AFB smear, and TNF-α levels can be useful as an alternative biomarker for the evaluation of the severity of pulmonary tuberculosis.

**Acknowledgments:** We would like to thank the Directorate of the Center for Chest and Respiratory Diseases / Erbil and the laboratory staff for their cooperation in carrying out this study.

**Financial support and sponsorship:** Nil.

**Conflicts of interest:** There are no conflicts of interest.

**Ethical Clearance:** Taken from both health and higher education and scientific research ministries in Iraq.

**References**


Original article
Effect of Resveratrol in Expression of Caspase-7 and Retinal Ganglion Cells in a Rat Model With Traumatic Optic Neuropathy

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Abstract

Objective: To analyze the effect of resveratrol to the expression of caspase-7 and density of retinal ganglion cells in a rat model with Traumatic Optic Neuropathy (TON) compared to control.

Methods: This is an experimental study using Wistar rats. Samples were divided into four groups. One normal control group, 1 TON control group and 2 TON resveratrol groups. TON induced by clamping the optic nerve retrobulbar with Hartmann Mosquito 2,5 inch. Resveratrol was given 10 mg/Kg and 20 mg/Kg in two resveratrol groups. Enucleation was performed 1 day after to evaluate the expression of Caspase-7 by immunohistochemistry and density of retinal ganglion cell by hematoxicilin eosin staining.

Results: Caspase-7 expression was lowest in the resveratrol 20 mg/kg treatment group (4.00 ± 2.00) compared to resveratrol 10 mg/kg treatment group (6.00±2.23), TON control group(6.00 ± 6.78) and normal control group(7.20 ± 2.58). Retinal ganglion cell density was highest in the resveratrol 10 mg/kg treatment group (27.23±2.75) compared to resveratrol 20 mg/kg treatment group (24.89±5.83), normal control group(24.19±2.39) and the lowest in TON control group (22.96±4.62). Based on the Pearson correlation test, it was found that there was no correlation between Caspase-7 expression and retinal ganglion cell density in a rat model with TON (p: 0.178; r = 0.314)

Conclusions: Resveratrol administration shows effect in lowering apoptosis marker caspase-7 and preserving retinal ganglion cell in traumatic optic neuropathy.

Keywords: Caspase-7, Traumatic optic neuropathy, Resveratrol, Retinal ganglion cell

Introduction

Retinal ganglion cell (RGC) death can occur due to apoptosis and necrosis mechanisms. Apoptosis is a cellular mechanism for programmed cell death but can be triggered by several factors including ischemia, toxic substances, and radiation1. Traumatic optic neuropathy (TON) is a disorder of the optic nerve caused by an acute injury with a clinical picture of a sudden decrease in visual acuity2.

The pathophysiology of TON involves several factors that are associated with the concept of primary injury and secondary injury that initiates retinal ganglion cell death2. Primary injury generally results from direct injury to the optic nerve, either compression or damage of the tissue, whereas secondary injury is a mechanism of optic nerve damage caused by inflammation or vascular disorders1.

Apoptosis can be lead by actvation of caspase. Caspase is a very interesting area of research on developmental disorders, cancer, infections, and
degenerative diseases. To date, two types of caspases have been defined, namely the caspase initiator and the effector / caspase executioner. As mentioned earlier, caspase-8 and -9 are initiator caspases while caspase 3 is effector caspases. Furthermore, other caspases such as caspase-2, -10, and -11 are included in caspase initiators while caspase-6 and -7 are included in the effector category. Still very little is known about caspase-7 in apoptosis of RGC. Caspase-7 was generally believed not exist in central nervous system. It was speculated that caspase-7 was acting as a redundant version of caspase-3 in apoptosis cascade. However, it was found that caspase-7 cleaves substrate different from caspase-3. Several non ocular studies suggest that caspase-7 play critical role in apoptosis and normal development. Decreased in caspase-7 protect the optic nerve from RGC death.

With apoptosis in research, the profound therapeutic potential of apoptosis has enabled researchers to develop promising therapeutic solutions that focus on the voluntary death of aberrant cells. A spectrum of drugs and therapies that exploit apoptosis has been shown to be effective against disease. Resveratrol (3,5,4-trihydroxystilbene) is a natural polyphenol found in grapes, red wine, berries and other plants. It has been identified as an inhibitor of carcinogenesis, cardiovascular diseases, neurodegenerative and aging. Many studies showed resveratrol effect on eye and related disorders. Similar effect in cancer, cardiovascular and neurodegenerative diseases, also seen in eye disorder including anti-oxidative, anti-apoptosis, anti-tumorogenic, anti-inflammation and anti-angiogenic.

Resveratrol can protect retina by inhibiting inflammation biomarkers interleukin-6 (IL-6) and interleukin-8 (IL-8), by transforming growth factor-β1 (TGF-β1), cyclooxygenase-2 (COX-2), and through VEGF accumulation. Resveratrol is known to activate sirtuins. The SIRT1 activating compounds promote longevity in different species and provide protective effects against acute or chronic neurodegenerative diseases, including retinal injury. Resveratrol prevented neuronal loss and delayed the visual decline.

Thus our study was aim to analyze the effect of resveratrol to the expression of caspase-7 and density of retinal ganglion cells in a rat model with Traumatic Optic Neuropathy.

**Material and Method**

This is an experimental study using rats. The research was carried out in the animal testing laboratory and the Anatomical Pathology Laboratory of the RSPTN UNHAS Makassar. The sample size was 24 rats.

**Work procedures**

a) Twenty-four rats were divided into 4 groups (6 per group), 1 normal control group without treatment, 1 TON control group without therapy and 2 groups of TON treated with resveratrol (10mg / kgBW and 20mg / kgBW).

b) TON induction is performed by clamping the optic nerve retrobulbar with Hartmann Mosquito 2,5 inch for 15 seconds until afferent pupillary response was negative.

c) Resveratrol was given post-induction orally at a dose of 10 and 20mg / kgBW using oral gavage to ensure drug distribution according to the expected dose.

d) The examination is carried out after going through the therapy interval. The induced eyeball then enucleated and prepared in a paraffin block with a sagittal cut and focuses on the inferior area of the optic nerve.

**Histopathological examination;**

Enucleated eyes were fixed with formaldehyde, embedded in paraffin and cut in 5µm thick section. For routine histological analysis. Section were stained with hematoxylin and eosin (H&E) and examined with light microscope. The number of retinal ganglion cell nuclei was counted in 40x magnification. The amount obtained is calculated per field of view and the average is calculated.

**Immunohistochemistry examination;**

a) Deparafinized the dried preparations with xylene 2 times (5 minutes each) and rehydrated with alcohol 96%, alcohol 80% and alcohol 70% (5 minutes each). Then wased for 5 minutes.

b) Put the preparation in TRS solution, and heated in microwave for 20 minutes. After cooling down, washed with PBS 2 times (5 minutes each).
c) Tissue margins were marked. Peroxide block was done for 15 minutes. Washed with PBS and put in protein block for 5 minutes and washed again in PBS twice (5 minutes each).

d) Antibody caspase-7 (Invitrogen) monoclonal antibody were diluted 1/100 and incubated in -20°C.

e) The preparation then washed with PBS twice (5 minutes each) and put in Ultratek anti-polyvalent (ScyTek) for 10 minutes.

f) The preparation then washed with PBS twice (5 minutes each) and put in Ultratek HRP (ScyTek) for 10 minutes. Then washed again twice (5 minutes each).

g) Preparation was incubated with chromogen Diaminobenzidine (DAB) and washed with PBS twice (5 minutes each), and soaked in hematoxylin solution for 5 minutes.

h) Preparation washed in running water. And dehydrated with alcohol 70%, alcohol 80%, alcohol 96% and clearing with xylol I and II 5 minutes each.

i) Slide then dried and prepared on the object glass.

j) The preparations were read under a light microscope. The number of caspase-7 expression based on brownish staining in sitoplasms and was counted in 40x magnification. The amount obtained is calculated per field of view and the average is calculated.

Data Analysis

All data obtained were processed using the SPSS program and analyzed with a significance level of ≤ 0.05. Independent t-test was used to compare two independent groups. Pearson’s correlation test was used to see the relationship between two different groups.

Result

Expression of caspase-7

Caspase-7 expression was lowest in the resveratrol 20 mg/kg treatment group (4.00 ± 2.00) compared to resveratrol 10 mg/kg treatment group (6.00±2.23), TON control group(6.00 ± 6.78) and normal control group(7.20 ± 2.58) (table 1). Figure 1 showed immunohistochemical staining of the rat retinal ganglion cell with caspase-7 antibody. The effect of resveratrol to the expression of caspase-7 in rat with TON showed that treatment with resveratrol decreased the expression of caspase-7 compared to TON control and normal control, treatment with higher dose (20 mg/kg) showed lower expression compared to 10 mg/kg dose. Although there was not significantly different (Table 2).

Figure 1

Microscopic description of caspase-7 expression in retinal ganglion cell (arrow) with immunohistochemistry.

Retinal ganglion cell density
Retinal ganglion cell density was highest in the resveratrol 10 mg/kg treatment group (27.23±2.75) compared to resveratrol 20 mg/kg treatment group (24.89±5.83), normal control group(24.19±2.39) and the lowest in TON control group (22.96±4.62) (table 1). Figure 2 showed retinal ganglion cell with hematoxylin eosin staining.

![Figure 2: microscopic retinal ganglion cells with H&E staining on (a) normal control, (b) TON control, (c) resveratrol TON mouse 10mg / kgBW, (d) resveratrol TON mouse 20mg / kgBb)](image)

**Table 1: Expression of caspase-7 and retinal ganglion cell density**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean±SD Expression of caspase-7</th>
<th>Mean±SD retinal ganglion cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>5</td>
<td>7.20±2.58</td>
<td>24.19±2.39</td>
</tr>
<tr>
<td>TON control</td>
<td>5</td>
<td>6.00±6.78</td>
<td>22.96±4.62</td>
</tr>
<tr>
<td>Resveratrol dosis 10 mg/kg</td>
<td>5</td>
<td>6.00±2.23</td>
<td>27.23±2.75</td>
</tr>
<tr>
<td>Resveratrol dosis 20 mg/kg</td>
<td>5</td>
<td>4.00±2.00</td>
<td>24.89±5.83</td>
</tr>
</tbody>
</table>
### Table 2: Differences in Expression of Caspase-7 and retinal ganglion cell density in Rat Models with TON between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Caspase-7 (Mean±SD)</th>
<th>Difference (P value)</th>
<th>Value (Mean±SD)</th>
<th>Difference (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TON control</td>
<td>5</td>
<td>6.00±6.78</td>
<td></td>
<td>22.96±4.62</td>
<td>1.22±2.32</td>
</tr>
<tr>
<td>Normal Control</td>
<td>5</td>
<td>7.20±2.58</td>
<td></td>
<td>24.19±2.39</td>
<td></td>
</tr>
<tr>
<td>TON control</td>
<td>5</td>
<td>6.00±6.78</td>
<td>0.00±3.19</td>
<td>22.96±4.62</td>
<td>4.27±2.40</td>
</tr>
<tr>
<td>Resveratrol 10 mg/kg</td>
<td>5</td>
<td>6.00±2.23</td>
<td></td>
<td>27.23±2.75</td>
<td></td>
</tr>
<tr>
<td>TON control</td>
<td>5</td>
<td>6.00±6.78</td>
<td>2.00±3.16</td>
<td>22.96±4.62</td>
<td>1.93±3.32</td>
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<tr>
<td>Resveratrol 20 mg/kg</td>
<td>5</td>
<td>4.00±2.00</td>
<td></td>
<td>24.89±5.83</td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>5</td>
<td>7.20±2.58</td>
<td>1.20±1.53</td>
<td>24.19±2.39</td>
<td>3.04±1.63</td>
</tr>
<tr>
<td>Resveratrol 10 mg/kg</td>
<td>5</td>
<td>6.00±2.23</td>
<td></td>
<td>27.23±2.75</td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>5</td>
<td>7.20±2.58</td>
<td>3.20±2.46</td>
<td>24.19±2.39</td>
<td>0.70±2.81</td>
</tr>
<tr>
<td>Resveratrol 20 mg/kg</td>
<td>5</td>
<td>4.00±2.00</td>
<td></td>
<td>24.89±5.83</td>
<td></td>
</tr>
<tr>
<td>Resveratrol 10 mg/kg</td>
<td>5</td>
<td>6.00±2.23</td>
<td>2.00±1.34</td>
<td>27.23±2.75</td>
<td>2.32±2.88</td>
</tr>
<tr>
<td>Resveratrol 20 mg/kg</td>
<td>5</td>
<td>4.00±2.00</td>
<td></td>
<td>24.89±5.83</td>
<td></td>
</tr>
</tbody>
</table>

**Independent T test**

Based on the results of the Pearson correlation test, it was found that there was no correlation between Caspase-7 expression and retinal ganglion cell density in a rat model with TON (p: 0.178; T= 0.314)
Table 3: Correlation Of Caspase-7 Expression And Retinal Ganglion Cell Density On Rat Model With TON

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-7 Levels Vs Retinal Ganglion Cells</td>
<td>0.314</td>
</tr>
</tbody>
</table>

### Discussion

#### Effectivity of resveratrol on expression of caspase-7 in rat with TON

The results of this study showed that there was no difference in the expression of Caspase-7 in rats with TON between the TON control group and the normal control group, resveratrol at a dose of 10mg / kg, and resveratrol at a dose of 20mg / kg (p> 0.05). However, based on the average number, the levels of caspase-7 were at the lowest in the 20mg / kgBW resveratrol group compared to the other groups. This implied that resveratrol can decrease the expression or caspase-7 in TON rats compared to controls.

Resveratrol has an inhibitory effect on cell continuity signals by downregulating survivin, one of the inhibitors of apoptosis proteins (IAPs). In general, apoptosis will trigger caspase activation. IAPs inhibit apoptosis by directly blocking activation of caspase-3 and -7 and caspase-9. This is in accordance with the results of our study which showed that TON rats given resveratrol had lower caspase-7 expression than normal control and TON control mice, and caspase-7 expression in TON mice given 20 mg / KgBW of resveratrol was lower than TON mice given a dose of 10 mg / kgBW. Thus showed the effect of different dosages on caspase-7 expression. Although statistically there was no significant difference.

The evaluated the role of resveratrol in mitochondrial biogenesis in inhibiting apoptosis in retinal ganglion cells. Resveratrol therapy is thought to inhibit the apoptosis process, maintain mitochondrial membrane potential, reduce caspase and reduce the release of cytochrome C thereby maintaining cell continuity. In Parkinson’s disease, low-dose resveratrol (5 μM) was thought to reduce dopamine-induced cell death in neuroblastoma cells by activating the antiapoptotic factor Bcl-2 and inhibiting caspase-3/7. Resveratrol’s effect on the pancreatic cancer cell renewal system by activating caspase-3 and -7 and inhibiting the expression of Bcl-2 and XIAP in cancer stem cells.

Apart from a role in apoptosis, caspase-7 also has a role in inflammation. For example, low mortality rates in mice low in caspase-7 were associated with significant protection against salivary cell death. The role of caspase-7 in apoptosis and inflammation indicates its function in cell death and / or inflammation that contributes to pathological conditions including neurodegenerative diseases.

On the other hand, this study showed the highest caspase-7 expression in normal control mice. This implied the role of caspase was not only in the regulation of apoptosis, but also in the phenomenon of non-apoptotic cells. And caspase-7 has a function in the progression of cell mitosis. This stated that caspase-3 and -7 are functionally present in cells in excessive amounts. This is also consistent with research.

#### Effectivity of resveratrol on retinal ganglion cell density in rat with TON

This study shows the effect of resveratrol on retinal ganglion cell density in TON rats at the highest dose of 10 mg / kgBW compared to the dose of 20mg / kgBW. This is probably due to the bioavailability of resveratrol in experimental animals. Resveratrol is very easily absorbed in humans and experimental animals but is quickly metabolized into sulfo- or gluco- or hydrogen derivatives, which will then be excreted in the urine. Resveratrol, which was taken orally at a single dose (25 mg = 110 μmol), the absorption was around 70%, with the concentration of resveratrol and its metabolites peaking at 2 μm after 1 hour.
Price et al found that low and moderate doses of resveratrol were required by AMPK activation and increased NAD +. However, at high doses, this effect is independent, indicating that the mechanism is dose dependent. Resveratrol shows its effectiveness even at low concentrations. The specific dose of resveratrol required for maximum effect is unknown. With regard to the method of administration of resveratrol therapy orally, several studies in mice have indicated that after oral administration of resveratrol, there is significant bioavailability in the cardiovascular system and binding to the kidneys and liver. Another study using mice, confirmed the same with mice regarding their bioavailability. It can even be said that resveratrol and its various derivatives can be found in various organs.13,14

This study showed no relationship between caspase-7 and retinal ganglion cell thickness in TON mice. This against previous studies which showed that caspase-7 has an important role in retinal ganglion cell survival in conditions of optic nerve injury, where the absence of caspase-7 protects against loss of retinal ganglion cells. However, this study also explains that caspase-7 is not the only mediator in the pathology of the injured optic nerve, caspase-3 may also plays a role.15

Conclusion

Resveratrol administration shows effect in lowering apoptosis marker caspase-7 and preserving retinal ganglion cell in traumatic optic neuropathy model in rat.

Source of Funding - Self-funding

Conflict of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 317 / UN4.6.4.5.31 // PP36 / 2020), and all research subjects give written informed consent.

References

The Effect of Ascorbic Acid and Selenium intake on serum Cortisol in Rats Under Restraint Stress

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¹Research Scholar; Physiology and Pharmacology Department/ College of Veterinary Medicine/ University of Kufa, ²Professor, Basic Medical Sciences Department/ College of Nursing/ University of Kufa, Iraq

Abstract

Over the recent years, vitamin C and selenium have been a clear line of defense against the effects of stress. The goal of the study is to indicate the effect of restraint stress in rats on the serum levels of cortisol, the role of vitamin C and selenium supplement in stress relief by determining their effect on serum cortisol. Forty Wistar rats were divided randomly into four equal groups (n=10), all the animals except negative control exposed to restraint stress for six hours a day. The first group were supplemented with vitamin C in a dose of (50 mg/kg bw/day) orally, the second included selenium in a dose of (0.02 µg/kg bw/day) orally, the control included ten rats. Blood was collected on 20th days, and serum cortisol were measured by enzyme-linked immunosorbent assay (ELISA). Results showed that supplement of rats with vitamin C had high significant increase in serum cortisol in stressed rat (14.65 ± 5) compared to those with no stress (8.77 ± 4) which constitute the negative control. The study also revealed that supplement of rats with vitamin C, Selenium and (vitamin C + Selenium) have highly significantly increased serum cortisol level to be (10.42 ± 3.78), (10.29 ± 4.54) and (12.77 ± 3.57) respectively compared to stressed rats. It is concluded that chronic stress restriction can result in cumulative initiation of cortisol secretion in rats. Intake of vitamin C can relieve stress by increasing decreasing cortisol.

Keywords: restraint stress, cortisol, vitamin C, selenium.

Introduction

The World Health Organization has considered stress to be the “Health Epidemic of the 21st Century” and is estimated to cost American businesses as much as $300 billion a year (¹). The effect of stress on our physical and emotional health can be devastating. More than 50 percent of individuals in the USA study felt that stress had a negative impact on productivity at work. Between 1983 and 2009, stress levels in all demographic groups in the United States increased by 10–30 per cent (²).

The majority of people experience certain form of stress at any given time. The term stress was used to describe a number of negative feelings and reactions which follow hazard or challenge cases. But the reactions to stress are not all negative. In fact, life requires a certain amount of stress. For example, childbirth is one of the most stressful events of life. The high levels of hormones released during birth, which are also involved in the response to stress, are thought to prepare the newborn baby to adapt to the challenges of life outside the womb (³).

Several studies indicate the effect of stress on the economy; there is clear evidence of economic stress posing a risk to mental health and reduces life satisfaction and behavioral alteration (²). Stress does not only directly hamper well-being, but also negative effects on other areas of life which lead to economic pressures and economic hardship. Vitamin C is also believed to be involved in anxiety, stress, depression, fatigue, and mood (⁴).
Oral vitamin C supplementation has been hypothesized to elevate morale as well as decrease depression and anxiety. By its antidepressant role, too, vitamin C can play antioxidant properties (5). In older people, vitamin C status has been associated with increased symptoms of depression following acute illness. In addition, the importance of vitamin C in decreasing anxiety caused by stress. Anxiety is an adaptive reaction to unknown danger, but when disproportionate to danger, it is pathological and continues beyond the presence of the stressor. Another research showed that dietary vitamin C supplementation (1000 mg/day) along with vitamins (A and E) resulted in a substantial reduction in depression scores over a 6-week period (6).

Another anti-stress element is selenium (Se); it has been shown to have a beneficial effect on mood of the selenium stage, at least if selenium is small. Low selenium status in studies has been linked despite a slightly greater frequency of depression and other adverse mood disorders such as anxiety, frustration, and selenium supplementation, mood tends to be improving (7). In the US report, high doses of selenium (226.5 μg per day) and overall mood disruption were significantly improved in clearheaded / confused, confident / unsure, and compound / anxious sub-scores. Similar findings were obtained in a double-blind cross over test conducted in the UK, where a 100 μg selenium supplement significantly reduced anxiety, fatigue and depression (8).

**Material and Methods**

It was experimental study, conducted March 2020 to the end of April 2020 on department of Physiology. Forty fertile adult’s albino rats (Rattus norvegicus) were used in the present study, their ages average (10-12), the weight range was (150-300) gm, the animals were housed in the animal house in a typical situation, in well ventilated wire-plastic cages with dimensions design cages (50× 35 × 15 cm) with metal covers and containing bedding of wood shaving which was changed once per daily. The animals were maintained under controlled environment about 12-hour light and 12-hour dark with (degree of temperature 22-26 °C) and exact circumstances to the normal laboratory nutrition with profitable diet (pellets) and water provided to animals through the all-time of the experiment. For two weeks before the experiment started, to adapt rats to the new environment none of the rats had any clinically obvious contagions, the rats were divided into four groups (A, B, C and D) with five rats for each cage.

Forty adult (male and female) lab rats were divided randomly in to four equal groups as following: -

First group: included ten rats (five male and five female) exposed to restraint stress for six hours a day, and supplemented with vitamin C in a dose of (7.2 mg / day) orally by gavage.

Second group: included ten rats (five male and five female) exposed to restraint stress for six hours a day, and supplemented with selenium in a dose of (0.02 μg / day) orally by gavage.

Third group: included ten rats (five male and five female) exposed to restraint stress for five hours, and supplemented with vitamin C in a dose of (7.2 mg/ day) + Selenium in a dose of (0.02μg /day) orally by gavage.

Fourth group: included five rats (three male and two female) rats exposed to restraint stress for five hours a day and given 2 ml normal saline orally by gavage and served as positive control (PC), and five other rats (three male and two female) without exposed to restraint stress, and given 2 ml normal saline orally by gavage and served as negative control (NC).

Rats were placed in the restraint cage used to produce restrain stress in a glass container (12×5 cm), for six-hour a day (9). The rats were exposed to stress between 08:30 AM and 14:30 PM for twenty days of the experiment as seen in figure (3-2) and movement of the rats was highly restricted as they are in the restraint container, negative control was not put in the restraint container all the period of experiment (10).

**Results**

All animals in the study were monitored for their feed and well-being and found healthy and active throughout the study period.

The values and statistical difference of serum cortisol at the twentieth day of experiment between rat groups are showed in table (1). According to this table, serum cortisol level has shown a high significant increase (P< 0.01) in stressed rat (14.65 ± 5) compared to those with no stress (8.77 ± 4) which constitute the
negative control.

The same table shows that supplement of rats with vitamin C, Selenium and (vitamin C + Selenium) have highly significantly (P< 0.01) increased serum cortisol level to be (10.42 ± 3.78), (10.29 ± 4.54) and (12.77 ± 3.57) respectively compared to stressed rats.

Table (1) : Differences in serum Cortisol level among rat groups at the twentieth day of experiment.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Total Number</th>
<th>Serum Cortisol (ng/dl) M ± SD</th>
<th>F test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (50 mg/kg)</td>
<td>10</td>
<td>10.42 ± 3.78 B</td>
<td></td>
</tr>
<tr>
<td>Selenium (0.02 mg/kg)</td>
<td>10</td>
<td>10.29 ± 4.54 B</td>
<td></td>
</tr>
<tr>
<td>Vitamin C + Selenium</td>
<td>10</td>
<td>12.77 ± 3.57 AB</td>
<td>2.66 (0.04)</td>
</tr>
<tr>
<td>Negative Control (without stress)</td>
<td>5</td>
<td>8.77 ± 4 B</td>
<td></td>
</tr>
<tr>
<td>Positive Control (with stress)</td>
<td>5</td>
<td>14.65 ± 5 A</td>
<td></td>
</tr>
</tbody>
</table>

A, B : Different letters show high significant difference at P< 0.01

**Discussion**

Stress is a well known health problem that negatively affects physical and emotional status of individuals and the economy of populations and countries (1). Physiologically, a wide range of hormones can be modulated in response to stress. For example, stress enhances the secretion of glucocorticoids, catecholamines, growth hormone and prolactin. Such physiological changes lead to mobilizing energy sources and tailoring individuals to their current circumstances (11). Importantly, cortisol. Represents the corner stone in psychological stress response, which in turn controls the systemic stress response components hence, cortisol is a main contributor in long-term homeostasis (12).

Consistent with the reported data, our findings showed that cortisol level is elevated in response stress as we found a significantly elevated level of cortisol in animals subjected to stress when compared to animals in the negative control group. The mechanism by which stress induces the release of cortisol. For example, blood samples collected from stressed rat showed increased ACTH/endorphins ratio. Elevated level of ACTH is significant characteristic of response to stress (13). Chronic use of glucocorticoid and stress induced release can modulate the activity of a wide range intercellular molecules resulting in altered cell homeostasis. It is suggested that stress can alter the response of the pituitary-adrenal axis to stress resulting in altered peptide processing or ACTH being selectively released (12). Our findings showed that vitamin C administration resulted in significantly decreased serum level of cortisol in stressed animals when compared to animals in the negative control group. Although it is studied in various models and study designs, several studies have shown a similar effect of vitamin C. For example, found that ascorbic acid (3000 mg a day) can enhance blood pressure and decrease salivary cortisol in patients exposed to acute psychological stress. Peters and colleagues observed that administration of 1000 mg of the vitamin C during a period of 8 days reduced post-race serum cortisol levels in athletes by 30 % reduction (14).
In terms of mechanisms by which vitamin C can reduce cortisol, several mechanisms have been proposed. Cortisol reducing effect of vitamin C may be attributed to its ability to inhibit enzymes responsible for steroidogenesis \(^{15}\). On an alternative way, cortisol released from the adrenals could be associated with oxidative stress-induced release of vitamin. Therefore, it is vitamin C supply with the vitamin may diminish its mobilization from its stores in the body, and consequently, attenuate the cortisol response \(^{16}\).

Responding to stress involves a wide range of biological processes by direct and or indirect actions of multiple systems. The hypothalamic pituitary-adrenocortical (HPA) axis plays a central biological response that is important to maintain homeostasis during dynamic changes. Both cortisol and DHEA are secreted and released by the HPA and serve as signaling molecules as a part within the hormonal cascade. In addition, they act as pleiotropic molecules centrally and peripherally \(^{17}\).

**Ethical Clearance**: Taken from University of Kufa ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


[16] Nussdorfer, G. G. and Mazzocchi, G. Immune-

Gene Expression and Serum IL-23 in Asthmatic Iraqi Children

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¹Analytics Technologist, ²Assist. Prof. Institute of Genetic Engineering And Biotechnology for Post Graduate Studies / Uni. of Baghdad

Abstract

Asthma is a non-communicable inflammatory airway disorder in which patients present with recurring bouts of breathlessness and wheezing. IL-23 is a proinflammatory cytokine its biological functions have been well known for the capability to enhance Th17 cell functions. Current study aimed to estimate the gene expression and serum level of IL-23 and to investigate whether IL-23 plays pivotal roles in the development of asthma in asthmatic Iraqi childhood. This study was conducted on two groups: seventy five asthma patients (27 female and 48 male) and twenty five apparently healthy as a control group (10 female and 15 male).The age of the samples ranged from (1-10) years old. Recruited from admitting the Central Teaching Hospital Pediatrics and Alzahra’a Center for Asthma Allergy in Baghdad during the period extended from October /2019 to last February/2020. Subject information’s were collected using a specific questionnaire form as a descriptive study; on the other hand, the present study was approved by the council of institute of genetic engineering and biotechnology for post graduate studies / University of Baghdad. The RNA was extracted from the blood sample of asthma patients and apparently healthy subjects by using TransZol Up Plus RNA Kit (blood). The acceptable purity of RNA in asthmatic patient is range between 1.84-1.99 and for apparently healthy group is range between 1.84-1.96. mRNA expression were determined by real time PCR assay and detect the concentration of IL-23 using ELISA technique. For IL-23 gene expression that showed the Ct of asthma patient group (22.82) and control group (23.33) and the 2-ΔΔCt of asthma patient group (5.35) and control group (3.70) and the fold of gene expression was statistically significantly (P≤0.05).

in the asthmatic group than healthy non asthmatic group that show in ratio (1.44:1.00). Human IL-23 concentration was estimated by ELISA, the results were statistically significant (P≤0.002), the level of IL-23 in asthma patient group (451.80 ± 91.80pg/ml) while the level of IL-23 in Control group(182.36 ± 58.21 pg/ml) as well as increase concentration of IL-23 in severe asthma patients than mild form asthma 726.91 ± 142.98 and 133.72 ± 79.56 respectively.

Key Word: Asthma Childhood, IL-23, qRT-PCR.

Introduction

Asthma of childhood is, a complex chronic disease, characterized by sever and chronic inflammation of airway, airflow limitation, wheezing, recurrent coughing, shortness of breath and chest tightness. Symptoms may occur several times in a day or week in affected individuals, and for some people become worse during physical activity or at night. During an asthma attack, the lining of the bronchial tubes swell, causing the airways to narrow and reducing the flow of air into and out of the lungs. Recurrent asthma symptoms frequently cause sleeplessness, daytime fatigue, reduced activity levels and school absenteeism. Asthma has a relatively low fatality rate compared to other chronic diseases¹. Asthma onset in children usually occurs before their fifth birthday. The majority of children with asthma are sensitive to household allergens and irritants, and they can benefit from a smoke-free, dust-free and pet-free environment ²,³. According to World Health Organization estimates that more than 339 million people had Asthma globally in 2016 ⁴. (IL-23) a member of the IL-12 family of cytokines, is a heterodimeric cytokine. It is composed of subunits p40 (shared with IL-12) and p19 (an IL-12 p35-related subunit) and is secreted by several types of immune cells, such as natural killer...
cells and dendritic cells. And is a protein consists of 189 amino acids with a molecular mass of 20730Da, and its gene lies on human chromosome 12q13.3, IL-23 may contribute to the differentiation of macrophages, but also antigen-induced Th2 cytokine production and eosinophil recruitment in the airways. IL-23 induces Memory T Cell Secretion of IL-17, the Memory (CD4+CD45RO) T cells secrete IL-17 in normal peripheral blood after activation in vitro. Addition of IL-23 alone to cultured memory (CD4+CD45RO) T cells induces a slight increase in IL-17 secretion. However, when other factors, such as activating anti-CD3/anti-CD28, are added together with IL-23, the level of IL-17 secreted from memory T cells significantly increases consistently as in, intracellular IL-17 mRNA levels significantly increase in these conditions.

The increase in IL-17 concentration is accompanied by the enhanced concentration of IL-23 which is a critical regulator of IL-17.

As to our knowledge, this is the first study about the genetic aspect of child asthma disease in genetic engineering and biotechnology institute so that. The present study aims to increase knowledge about the occurrence of Asthma in child in Iraq to determining the gene expression of IL-23 in patient by Real time PCR as well as determine serum level of IL-23 using ELISA technique.

Subjects, Materials and Methods

Study consist of two groups, Seventy Five patients (27 female and 48 male) and twenty five as apparently healthy subjects (control) and personal information such as: age, season, gender, family history, sensitivity, incidence intensity, onset of disease, other diseases, the samples were admitting the Central Teaching Hospital Pediatrics and Alzahra’a Center for Asthma and Allergy in Baghdad during the period between October /2019 to February /2020. The study design was approved by the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies/ University of Baghdad. Writing informed consents were obtained from all patients and apparently healthy control group; all patients were diagnosed according to clinical examination by a chest physician and selected according to the criteria of the global initiate of asthma.

Genomic RNA extraction and determine level IL-23 in serum

Three ml of peripheral venous blood samples were collected from the asthma patients and apparently healthy control using disposable latex gloves and syringes. Then,1 ml of blood was kept in EDTA anticoagulant tubes and then converted to 0.25 ml of EDTA blood in Eppendorf tubes that contain 1ml Trizol and freezer at -20°C to be a source for RNA extraction. The RNA was extracted from the samples of blood of asthma patients and apparently healthy subjects by using TransZol Up Plus RNA Kit (blood) company of kit (Transgen). Then, RNA concentration and purity were measured by nanodrop. The acceptable purity asthmatic patient of samples of RNA is range between 1.84-1.99 and for healthy group samples of RNA purity is range between 1.84-1.96. and level of IL-23 determining in serum of asthma patients and apparently healthy by using IL-23 ELISA kit, ELISA kit. MY BIOSOURSE COMPANY was used in this study.

cDNA synthesis for mRNA

Total RNA was reversely transcribed to complementary DNA (cDNA) using EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix. The procedure was carried out in a reaction volume of 20 μl according to the manufacturer’s instructions. The total RNA volume to be reversely transcribed was (20μl).

Real time PCR Primers for IL-23 Gene:

The primer sequence of this study.

Interleukin 23: Forward 5' AGTGGAAGTGGGCAGAGATTC-3' Reverse 5' CAGCAGCAACAGCAGCATTAC-3' GAPDH: Forward 5' TGAGAAGTATGACAACAGCC-3' Reverse 5' TCCTTCCACGATACCAAAG- Components of quantitative real-time PCR used in IL-23 (Transgen / China).
Real Time PCR Program

The expression levels of *(IL-23)*, and housekeeping genes *(GAPDH)* were estimated by RT-PCR To confirm the expression of target gene, quantitative real time RT-PCR SYBR Green assay was used. Primers sequences for *(IL-23)*, as housekeeping gene *(GAPDH)* were prepared according to 9. Optimal annealing temperature of qPCR reaction was found out after several try to 64˚C with a total volume of 50 μl. The reaction components are described in table (1).

### Table (1) Thermal profile of *IL23* gene expression

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activation</td>
<td>94°C</td>
<td>30 sec</td>
<td>Hold</td>
</tr>
<tr>
<td>Denature</td>
<td>94°C</td>
<td>5 sec</td>
<td>40</td>
</tr>
<tr>
<td>Anneal</td>
<td>64°C</td>
<td>20 sec</td>
<td></td>
</tr>
<tr>
<td>extend</td>
<td>72°C</td>
<td>20 sec</td>
<td></td>
</tr>
<tr>
<td>Dissociation</td>
<td>1 min /95°C-30 sec /60°C-30sec/95 °C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Real Time RT-PCR analysis of *IL-23* gene expression:

1. DCT

The expression ratio was calculated without a calibrator sample 2-DCT according to the following equation:

\[
DCT (test) = CT \text{ gene of interest (target, test)} - CT \text{ internal control}
\]

Finally, the expression ratio was calculated according to the formula

\[
2^{-\Delta CT} = \text{Normalized expression ratio}
\]

2. DD CT

To compare the transcript levels between different samples the \(2^{-\Delta DCt}\) method was used 10.

The CT of gene of interest was normalized to that of internal control gene. And the CT was calculated as the following formula:
DCT (test) = CT gene of interest (target, test) – CT internal control

DCT (calibrator) = CT gene of interest (target, calibrator) – CT internal control. The calibrator was chosen from the control samples.

CT values ≥ 38 were considered unreliable and neglected

The DCT of the test samples was normalized to the DCT of the calibrator:

DD CT was calculated according to the following equation:

DD CT=DCT (test)- DCT(calibrator)

Finally, the expression ratio was calculated according to the formula

2-DDCT = Normalized expression ratio.

**Determination of IL-23 titer using Enzyme-Linked Immunosorbent Assay kit (My biosoure/USA)**

**Statistical analysis**

According to¹¹. (SAS) Program was used to determine the fold of gene expression. Least significant difference –LSD test (Analysis of Variation-ANOVA) or T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

**Result and Discussion**

**RNA Extraction**

Total RNA was successfully extracted from all samples. The concentration of total RNA ranged from 83-188 ng/μl and from 81-182 ng/μl in healthy group (non asthmatic). And the purity of total RNA samples ranged from 1.84-1.99 ng/μl in the patients asthmatic group, and from 1.84-1.96 ng/μl in the healthy non asthmatic group. With p-value 0.74.

There was no significant difference between the concentration of the total RNA of the two study groups, p=0.366. There was no significant difference between the RNA purity of the two study groups as well.

**Real time PCR quantification of IL-23 Expression:**

The mean Ct value of IL-23 cDNA amplification were (22.82) in the patients asthmatic group, and the mean Ct healthy non asthmatic group were (23.33). The results are shown in table (2). There was a significant difference in the mean Ct values between the different study groups ((P≤0.05).

This depends on normalization of Ct values calculating the ΔCt which is the difference between the mean Ct values of replica of IL-23cDNA amplification of each single case and that of the GAPDH.

Table (2) shows the mean of ΔCt (normalization Ct values) of each study group. ΔCt means in asthmatic patient group(0.57). And healthy non asthmatic group was (1.1). A significant difference was noticed between the study groups (P≤0.05)

Results of 2-ΔCt revealed significantly higher results for the asthmatic patient group from the healthy non asthmatic group (P≤0.05), mean of 2-ΔCt for asthmatic patient group (0.673) while In the healthy non asthmatic group a mean of 2-ΔCt was (0.466).

To calculate the gene expression folds in relation to the housekeeping genes the result of 2-ΔCt of each group was measured in relation to that of Healthy non asthmatic group .The results are shown in table (2). The fold of gene expression in asthmatic patient group was higher than Healthy non asthmatic group in 1.44 times. While the fold number in healthy non asthmatic group was1.00 times. as in table (2). These results indicate a significantly increase expression of B-actin gene in these groups.
Table (2): Fold of IL-23 expression Depending on 2-ΔCt Method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Means Ct of IL-23</th>
<th>Means Ct of GAPDH</th>
<th>ΔCt (Means Ct of IL-23 - Means Ct of GAPDH)</th>
<th>2-ΔCt experimental group/Control group</th>
<th>Fold of gene expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma patient group</td>
<td>22.82</td>
<td>22.25</td>
<td>0.57</td>
<td>0.673</td>
<td>0.673/0.466</td>
</tr>
<tr>
<td>Healthy group</td>
<td>23.33</td>
<td>22.23</td>
<td>1.1</td>
<td>0.466</td>
<td>0.466/0.466</td>
</tr>
<tr>
<td>T-test</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>P-value</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

* (P≤0.05).

As shown in table (3), the mean of 2-ΔΔCt values of asthmatic patient group and Healthy non asthmatic group it was (5.35) and (3.70) respectively. There was a significant difference between these groups regarding the mean 2-ΔΔCt, (p≤0.05).

Table (3): Fold of IL-23 expression Depending on 2-ΔΔCt Method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Means Ct of IL-23</th>
<th>Means Ct of GAPDH</th>
<th>ΔCt (Means Ct of IL-23 - Means Ct of GAPDH)</th>
<th>Mean ΔCt Calibrator (ctIL23-ctGAPDH)</th>
<th>ΔΔCt</th>
<th>2-ΔΔCt</th>
<th>experimental group/Control group</th>
<th>Fold of gene expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma patient group</td>
<td>22.82</td>
<td>22.25</td>
<td>0.57</td>
<td>2.99</td>
<td>-2.42</td>
<td>5.35</td>
<td>5.35/3.70</td>
<td>1.44 ± 0.07</td>
</tr>
<tr>
<td>Healthy group</td>
<td>23.33</td>
<td>22.23</td>
<td>1.1</td>
<td>2.99</td>
<td>-1.89</td>
<td>3.70</td>
<td>3.70/3.70</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>T-test</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.288 *</td>
</tr>
<tr>
<td>P-value</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.0372</td>
</tr>
</tbody>
</table>

* (P≤0.05).

When calculating gene expression it was significantly higher in asthmatic patient group than Healthy non asthmatic group 1.44 times. as shown in table (3). The above results demonstrate the significant gene expression in Healthy non asthmatic group.

The mean Ct values in healthy non asthmatic group were higher than those of asthmatic patient group .This is important in reflecting the original mRNAs present in the samples. It is evident from these results that the patients group is associated with the highest copy number of mRNAs reflecting its higher expression.
The results show convergence of Ct values between asthmatic patient group and healthy non asthmatic group it is important evidence that IL-23 gene expression so it is possible to use IL-23 gene as a biomarker for the early detection of asthma disease.

These results agreed with\textsuperscript {11}. Their result indicate IL-23 levels were higher in severe asthmatics than in control group Thus, it is presumably that IL-23 could be a suitable marker of allergic inflammation in asthma.

Figures (1),(2) show the amplification plots and dissociation curves for IL-23.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{IL-23 amplification plots by qPCR Samples included all study groups. Ct values ranged from 21.97 to 23.75. The photograph was taken directly from Cephoidsmartcycler qPCR machine}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{IL-23 dissociation curves by qPCR Samples included all study groups. Melting temperature ranged from 76.78°C to 79.96°C. The photograph was taken directly from Cephoid smart cycler qPCR machine}
\end{figure}
Serological Study:

Concentrations of \( IL-23 \) in blood serum of asthmatic patient and control group, that show significant between asthmatic patients and control group \( (P \leq 0.002) \). The Mean ± SE of asthma Patients\((451.80 ± 91.80 \text{pg/ml}) \) and Mean ± SE of Control group\((182.36 ± 58.21 \text{pg/ml}) \).

This result is Similar to study 12. That measure the level of \( IL-23 \) in Seventy-eight asthmatic children and 40 healthy children by ELISA were evaluated \( IL-23 \) levels were higher in asthmatic than in healthy children.

And when Comparison level of cytokine \( (IL-23) \) between asthma patient in different forms asthma (sever and mild) and control group the result appear significantly between these groups \( (P \leq 0.002) \), the concentration of \( IL-23 \) in sever asthma patient \((726.91 ± 142.98) \), mild asthma patient \((133.72 ± 79.56) \) and control group\((182.36 ± 58.22) \) Table (10), figure (3). The concentration of \( IL-23 \) increase with severity form asthma more than mild form and these result identical with gene expression result that previously offered. This study is identical with several studies13,14.

![Figure (3): Comparison between patients (Sever, Mild) and control groups in concentration of \( IL-23 \).](image)

**Conclusion**

Study revealed the an increase \( IL-23 \) mRNA expressions and serum \( IL-23 \) concentrations in children with severe asthma compared to that with mild of the disease and control group.

**Recommendations**: Study the gene expression and level of \( IL-23 \) in asthma adult.

**Acknowledgements**: We are grateful to the persons for their involvement in this study.

**Ethical Clearance**: The principles and the experimental protocol in this study was approved by the medico – legal directorate, Ministry of Health, Baghdad, Iraq.

**Source of Funding**: Self

**Conflict of Interest** - nil

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14. Rasul Sh, Güresir E, Vatter H, Muhammad S. Aneurysmal subarachnoid hemorrhage lead to systemic upregulation of IL-23/IL-17 inflammatory axis, Cytokine, 2017; 97: 96-103.
A Comparative Study to Evaluate the Effect of Ultrasonic Agitation on Push-Out Bond Strength of Three Root-End Filling Materials: An In Vitro Study

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Abstract

Aim: To evaluate the effect of ultrasonic agitation for retrograde biodceramic root repair, MTA and biodentine filling materials on push-out bond strength to dentine walls.

Materials and Methods: Ninety extracted human teeth with single straight roots were selected randomly. After disinfection and cleaning, the coronal portions were sectioned to standardize the root canal length at 15mm. following root canal shaping, obturation and apical roots resection, retrograde cavities were prepared. Teeth were categorized depending on the filling material used into three groups, 30 teeth each. Group A filled with bioceramic root repair material, B with MTA and C with Biodentine material. These groups were divided in to three subgroup (n=10). Subgroups:A1, B1, and C1 cavities were filled with the aid of condenser. A2, B2, and C2 with ultrasonic agitation of material for 30 seconds. A3, B3, and C3 with ultrasonic agitation of material for 60 seconds. Push-out bond strength test was done for the samples to specify bond strengths at the cement/dentin interfaces. The specimens were examined using a stereomicroscope to estimate the mode of failure. Data were statistically analyzed using two-way ANOVA and Bonferonni test.

Results: Ultrasonic agitations increase the bond strength for all cements. Biodentine™, PD™ MTAWhite and TotalFill® RRM™ were significantly increased (P<0.05). While, increasing the time of ultrasonic was statically non-significant among groups.

Conclusions: Ultrasonic agitation of root-ending filling materials showed increase in push-out bound strength to dentine walls.

Key word: Retrograde-filling, bioceramic, biodentine, MTA, ultrasonic agitation.

Introduction

Retrograde root canal filling following apicoectomy is an important procedure, filling of root-end cavity with an adequate retrograde filling material is important to prevent the invasion of bacteria and apical microleakage.[1] Numerous materials were developed as root-end filling materials such as mineral trioxide aggregate (MTA), which induce cementogenesis and has noncytotoxic effect. Consequently, currently MTA is used as a root-filling material. However, MTA showed some disadvantages: long setting time, difficult handling, discoloration, high cost and inadequate antibacterial qualities.[2] To overcome these problems, new root-filling materials have been developed, they are bioactive which means when they come in contact with vital tissues they facilitate apical repair and biomineralization.[3]

Biodentine has many similar properties when compared to MTA but with a faster setting time and better consistency, therefore it is considered as an alternative to MTA. The absence of calcium sulfate and calcium aluminates in Biodentine formulation that are known to give a longer setting time and decreased mechanical strength is the major difference between this new material and MTA calcium silicates.[4, 5] Other
materials such as bioceramics root repair materials are used in root-end fillings, due to their better properties such as decreased moisture sensitivity, insolubility, and tissue inductive properties, they are the materials of choice in endodontics treatment. The addition of silicate-based materials supposed to decrease setting times. Thus it was overcoming the drawbacks of MTA.[6]

Push-out bond strength test was done for the samples to specify bond strengths between dentin interfaces the retrograde filling materials. It is a mechanical test used to measure the resistance of a material to dislodgment and its adhesive property.[7] Ultrasonic energy may be employed to enhance the introduction of root-end filling within the root canal aiming to improve root canal filling. Therefore ultrasound may be suggested to be an auxiliary tool for filling material introduction in root-end cavities.[8, 9]

The aim of this study was to evaluate the effect of ultrasonic agitation for retrograde bioceramic root repair, MTA and biodentine filling materials on the push-out bond strength. The null hypothesis indicates that no differences among bond strength values for used materials and the mode of application in retrograde filling.

**Materials and Method**

Ninety extracted teeth were randomly selected from the department of maxillofacial dentistry, University of Baghdad, all teeth were extracted for the purpose of patients’ treatment, teeth were collected according to the following criteria: Permanent maxillary incisors with single roots that: completed the root formation, without any anatomic variations and have patent canals.[10]

Preoperative buccolingual and mesiodistal radiographs of each root of studied teeth were taken to confirm that there was no internal or external resorption or calcifications. Teeth were autoclaved and stored in 0.2% thymol solution until they were used.[11] The teeth were cleaned carefully for any calculus deposits with air scalar and polished pumice.[12] The coronal portions were removed using diamond disc (0.2mm thickness) to standardize the root canal (15mm in length).[8] Then, the pulp tissue was removed with a barbed broach and a size 10 k-file (DENTSPLY Maillefer, Ballaigues, Switzerland), the tip of the file was 1mm visible from the apical foramen.

The root canals were prepared to the desired shape with the ProTaper rotary files (DENTSPLY, Maillefer, and Ballaigues, Switzerland). The preparation was started with SX file and followed by S1, S2, F1, F2 and F3 files. Between each two files, the canal was rinsed with 2mL of 5.25% sodium hypochlorite, then the canal was irrigated by 2mL (EDTA) at 17% concentration (Dental Produits Dentaires SA, Switzerland) for three minutes, finally canals wear rinsed by 2ml of distilled water and then dried using paper points (ULTRADENT UT,USA). Gutta-percha points and zinc-phosphate cement (Adhesor, Spofa Dental) were used to fill the canals. For complete setting of the filling materials, the roots were stored in 100% humidity at 37°C for 2days.

Then specimens were embedded in acrylic resin, the coronal surface of the root was fixed with sticky wax to the dental surveyor to ensure accurate sectioning at right angle to the long axis of the roots. The roots were carefully sectioned at right angle to long axis of the tooth (3mm from the apex) under continuous water cooling using a diamond disk (0.2mm thickness). Then, the root was fixed in an apparatus to acquire a root-end cavities and to obtain parallel dentin walls,[13] root-end cavities measuring 1.5mm in diameter and 3mm in depth were prepared,[9] and filled by the aid of Micro-Apical placement System (Produits Dentaires, Switzerland).

The teeth divided into 3 groups according to the filling material used:

**Group A:30 teeth**, the retrograde cavities were filled with TotalFill® BC RRM™ (Total fill bioceramic root repair material, putty (jar2.5g) FKG-Dentaire SA,Switzerland), this having 3 subgroups (n=10):

- A1:10 cavities filled with the aid of condenser (Trinity, São Paulo, SP, Brazil).
- A2:10 cavities filled with ultrasonic agitation of material for 30seconds (15seconds in the mesio-distal direction and 15seconds in the bucco-lingual direction), using a mini Irrisonic tip which was the prototype of an ultrasonic tip, it was commercially produced.
- A3:10 cavities filled with ultrasonic agitation of material for 60seconds (30seconds in the mesio-distal direction and 30seconds in the bucco-lingual direction),
using the same tip as in group A2.

**Group B:** 30 teeth, the retrograde cavities were filled with PD™ MTA White (Produits Dentaires SA Rue des Béosquets 18, Switzerland), this having 3 subgroups ($n=10$):

- B1: 10 cavities filled with the aid of condenser.
- B2: 10 cavities filled with ultrasonic agitation of material as in subgroup A2.
- B3: 10 cavities filled with ultrasonic agitation of material as in subgroup A3.

**Group C:** 30 teeth, the retrograde cavities were filled with Biodentine™ (Septodont, Saint Maur de Fosses; France), this having 3 subgroups ($n=10$):

- C1: 10 cavities filled with the aid of condenser.
- C2: 10 cavities filled with ultrasonic agitation of material as in subgroup A2.
- C3: 10 cavities filled with ultrasonic agitation of material as in subgroup A3.

Radiographs were taken to confirm proper filling of the material. For complete setting of materials, all teeth were stored at 37°C and 100% humidity for 7 days. During all sectioning, Struers minitom cutting equipment (Copenhagen/Denmark) was used. The slices were determined with digital caliper and marker to obtain 2 mm cut. Sectioning was carried out with the diamond disc (0.2 mm thickness) and water coolant. All samples were examined after sectioning and any slice with oval shaped canal or voids within the sealer should be discarded.

Push-out bond strength test was done for the samples (to specify bond strengths at the cement/dentin interfaces) via cylindrical plunger mounted on Universal testing machine (Tinius, Olsen, UK) managed by computer software (Figure 1). The plunger used was 1.4 mm. Micro push-out testing was done at a crosshead speed of 0.5 mm/minute until failure occurred.

**Push-out strength data were determined in MPa according to the following formula:**

\[
\text{Push-out strength} = \frac{\text{force of dislodgment in Newton}}{\text{bonded surface area in mm}^2}
\]

The area ($\text{mm}^2$) under load was calculated by the cylinder lateral surface area formula:

\[
\text{bonding area} = 2 \times \pi \times r \times h
\]

Where $r$ is the radius of the preparation circumference, $\pi = 3.14$ and $h$ is the thicknesses of the root slice (2 mm).

Two-way ANOVA and Bonferonni test with software SPSS Version 21.0. (IBM, Armonk, NY, USA) were used to statistically analyze the data, at a level of significance ($P<0.05$).

**Results:**

Tests were done to the teeth and push-out bond strength values were measured in Mpa for all groups.

The result of the two-way ANOVA showed that the largest effect size on push-out bond strength was between subgroups of group B followed by group C and the least effect size was in group A (Table 1). Bonferonni-test was used (Table 2) to find the subgroups responsible for the difference.

ANOVA analysis of variance was used at level of significance (0.05) as in Table 3. In order to determine materials effect on push-out bond strength. The result showed that the largest effect of ultrasonic on push-out bond strength was between ultrasonic agitation of material subgroups for 60 seconds followed by subgroups of the aid of condenser and the least effect size was in subgroups of ultrasonic agitation of material for 60 seconds. Bonferonni-test was used (Table 4) to find the subgroups responsible for the difference.

The analysis of failure modes for push-out bond strength, mixed failure were the most failure patterns that noted in groups (Table 5).
Table 1: The effect of subgroups (based on cement application method) on push-out bond strength by using Two-way ANOVA test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TotalFill® BC RRM™)</td>
<td>65.783</td>
<td>2</td>
<td>32.891</td>
<td>6.151</td>
<td>0.006</td>
<td>0.262</td>
</tr>
<tr>
<td>(PD™ MTA White)</td>
<td>104.960</td>
<td>2</td>
<td>52.480</td>
<td>15.454</td>
<td>0.000</td>
<td>0.499 Large effect</td>
</tr>
<tr>
<td>(Biodentine™)</td>
<td>50.529</td>
<td>2</td>
<td>25.264</td>
<td>6.426</td>
<td>0.005</td>
<td>0.272</td>
</tr>
</tbody>
</table>

Table 2: Bonferroni test between subgroups for each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>(I) Subgroups</th>
<th>(J) Subgroups</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TotalFill® BC RRM™)</td>
<td>A1</td>
<td>A2</td>
<td>-2.687</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>A3</td>
<td>-3.453</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>A1</td>
<td>A3</td>
<td>-0.766</td>
<td>1.000</td>
</tr>
<tr>
<td>(PD™ MTA White)</td>
<td>B1</td>
<td>B2</td>
<td>-3.663</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>B3</td>
<td>-4.214</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>B3</td>
<td>-0.551</td>
<td>1.000</td>
</tr>
<tr>
<td>(Biodentine™)</td>
<td>C1</td>
<td>C2</td>
<td>-2.459</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>C3</td>
<td>-2.974</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>C3</td>
<td>-0.515</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Table 3: The effect of subgroups (based on cement type) on push-out bond strength by using Two-way ANOVA test

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aid of condenser</td>
<td>69.100</td>
<td>2</td>
<td>34.550</td>
<td>5.310</td>
<td>0.011</td>
<td>0.229</td>
</tr>
<tr>
<td>Ultrasonic 30 Sec</td>
<td>31.947</td>
<td>2</td>
<td>15.973</td>
<td>3.897</td>
<td>0.033</td>
<td>0.167</td>
</tr>
<tr>
<td>Ultrasonic 60 Sec</td>
<td>36.578</td>
<td>2</td>
<td>18.289</td>
<td>8.836</td>
<td>0.001</td>
<td>0.351 Large effect</td>
</tr>
</tbody>
</table>
### Table 4: Bonferroni test adjustment for multiple comparisons within subgroups

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>(I) Subgroups</th>
<th>(J) Subgroups</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aid of condenser</td>
<td>A1</td>
<td>B1</td>
<td>3.429</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1</td>
<td>0.473</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>C1</td>
<td>-2.956</td>
<td>0.046</td>
</tr>
<tr>
<td>Ultrasonic 30Sec</td>
<td>A2</td>
<td>B2</td>
<td>2.453</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2</td>
<td>0.701</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>C2</td>
<td>-1.752</td>
<td>0.190</td>
</tr>
<tr>
<td>Ultrasonic 60Sec</td>
<td>A3</td>
<td>B3</td>
<td>2.668</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3</td>
<td>0.952</td>
<td>0.451</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>C3</td>
<td>-1.716</td>
<td>0.038</td>
</tr>
</tbody>
</table>

### Table 5: Comparison of failure mode among groups

<table>
<thead>
<tr>
<th>Failure Mode</th>
<th>Total Fill</th>
<th>BC</th>
<th>RRM</th>
<th>MTA</th>
<th>Biodentine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
<td>B1</td>
<td>B2</td>
</tr>
<tr>
<td>Adhesive</td>
<td>10%</td>
<td>0%</td>
<td>0%</td>
<td>40%</td>
<td>20%</td>
</tr>
<tr>
<td>Cohesive</td>
<td>20%</td>
<td>30%</td>
<td>20%</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>Mixed</td>
<td>70%</td>
<td>70%</td>
<td>80%</td>
<td>60%</td>
<td>70%</td>
</tr>
</tbody>
</table>

**Figure 1:** A: Specimen fixed in acrylic resin. B: Specimen under vertical load.
Discussion

Surgical apicoectomy followed by root-end filling is a choice to avert tooth extraction while conventional endodontic therapy has failed. It is clinically very important to minimize the microleakage at the periapical area. For this reason, thorough understanding in the material ability to bond to the tooth structure help in the selection of the material and in the prediction of the procedure outcomes. In many studies, the push out test was commonly done to determine the bonding strength between the root-end filling materials and the dentinal walls.

According to this study, all root-end filling materials showed bonding at the apical region, it was found that Group A (TotalFill® BC RRM™) exhibited significantly higher bond strength followed by Group C (Biodentine) and Group B (MTA). Ultrasonic agitation enhanced the push-out bond strength of materials. Consequently, the null hypothesis was rejected. Ultrasonic of retrograde filling has been previously tested by a study concluded that ultrasonic agitation of retrograde filling enhanced the bond to the dentinal wall of the retrograde filling materials. This study tested if ultrasonic agitation of three cements during its insertion at different times increases its bond strength. This may be due to high adaptation of the cement to the dentin. Ultrasonic agitation of material exhibit significantly higher bond strength especially for TotalFill® BC RRM™ group but the large effect of agitation was found in PD™ MTA White group. Biodentine had higher bond strength values than that of MTA groups and this agree with Eren et al. Increasing the time of ultrasonic increase the bond strength but statically is non-significant for all groups. Ultrasonic effect showed good results in terms of intratubular sealers penetration. The dentine uptake was more prominent for Biodentine than MTA which increased depth of silicon and calcium penetration into dentin, when compared to MTA, Biodentine possess a better homogeneity and smaller particles. Subsequently, the better bonding strength of TotalFill® BC RRM™ to dentin in this study could be explained by silicon and calcium uptake by the dentin. And this agrees with other study reported by Kadić. et al.

All materials showed mixed failures predominantly, which was reported by other previous studies. Cement materials showed some cohesive failures weaknesses in material itself, also adhesive failures in the bonding with the dentinal walls were present. However, TotalFill® BC RRM™ shows significantly higher bonding strength in relation to MTA and non-significantly higher bonding strength in compared to Biodentine.

This study showed ultrasonic agitation improved the bonding strength and adaptation between the dentinal wall and the restoration which can improve the clinical outcomes of the retrograde root-end filling. Failures were observed in the tested materials were from the mixed type which are of both cohesive failure (weakness in the materials) and adhesive failure (bonding to the dentinal walls). Also it should be noted that all tests were done under perfect laboratory condition with samples that are carefully selected with specific criteria, therefore, any other in vivo environmental factors such as temperature and saliva also teeth variation were excluded and could have an impact on the bonding strength thus, more research under clinical conditions should be done to deeper understanding of this technique to assess the true interaction between the dentinal wall and the materials.

Conclusion

According to methodology and results, the ultrasonic of retrograde cement materials showed higher bonding strength to the dentinal walls and the large effect of agitation was found in PD™ MTA White group.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

References


2. Kumari S, Mittal A, Dadu S, Dhaundiyal A, Abraham A, Yendrembam B. Comparative evaluation of physical and chemical properties of


The Effect of Drinking Water Quality on Some Hematological Parameters in Female Laboratory Mice

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Abstract

The present study was designed to determine the effect of two sources of drinking water on some hematological parameters in female laboratory mice. The animals were divided into three groups: First group (control) it given a physiological solution, Second group: the RO water was given and third group: the river water was given.

Results: the results of the study showed a significant increase ($p \leq 0.05$) in the erythrocyte, leukocytes count, percentage of lymphocytes and granular cells, significant decrease in Hb concentration and PCV percentage in the second and third group when compared with the control group, while mean corpuscular volume insignificantly decrease in the second group and significantly increased in third group. Results also showed a significant increase in platelet count and monocytes percentage in the second group while it decreased in the third group.

Conclusion: we conclude from this study that the water source and its quality have a significant effect on the blood parameters of laboratory animals, and then influence on the animal’s health.

Keywords: Mice; Water; Hematological; RO; Pollution; Rivers.

Introduction

Water is the nerve of life and an important resource for humans, as it constitutes a large percentage of the content of the cell’s living matter in all organisms and a medium for biological reactions, as well as for this it contains many salts ions that causes the survive and continuation of organism in life (1).

As a result of water pollution, hundreds of millions of people around the world do not have access to an adequate supply of safe drinking water besides poor sanitation and hygiene, and polluted water causes the death of nearly 3.4 million cases annually, most of them are children and more than one billion people are still not they have access to good water sources despite the continuous efforts of governments, civil societies and the international community (2).

Consumption of water from poor sources along with the use of untreated water in agriculture is responsible for many disorders such as health damage to humans such as increased cases of diarrhea, for example Dokan river was the potential source of cholera outbreak in Sulaymaniyah (266 cases) and Kirkuk (160 confirmed cases) in October 2012 and is an example of the health risk of river water pollution as sources of drinking (3).

Monitoring and protecting water quality is an important issue, and the physical, chemical and biological characteristics of river water are important for assessing the effects that cause river water quality to deteriorate, and studying it is essential to water quality and reducing the effects of degradation (4).

Reverse osmosis water (RO) is a water purification process that uses a partially permeable membrane to remove unwanted molecules, ions from drinking water. In RO, an applied pressure is used for overcome osmotic pressuring a colligative property that is driven by chemical potential differences of the solvent, a thermodynamic parameter. RO can remove many types of suspended and dissolved chemical species as well as biological types (mainly bacteria) from water and is used in both industrial processes and the production of potable...
water. The result is that the solute is retained on the pressurized side of the membrane and the pure solvent is allowed to pass to the other side. To be selective, this membrane should not allow ions or large molecules through the holes, but should allow smaller components of the solution (such as H$_2$O) to pass freely (5).

**Material and Method**

**Animals preparation**

Eighteen healthy 10-12 weeks old female albino mice and weighing 22-25g were obtained from Thi-Qar University/College of Science. Mice were placed in a controlled temperature room (23-25) c° under a 12hour dark-12hour light cycle.

**Samples of water**

Water samples were collected from the Euphrates river in Nasiriyah / Dhi-Qar Governorate (the area located in front of the College of Education for Pure Science). Physical, chemical and biological characteristic were evaluated which have importance in determining the validity of the water whether it is river water or drinking water (6) as shown in table (1).

<table>
<thead>
<tr>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Cl</th>
<th>pH</th>
<th>ES</th>
<th>TDS</th>
<th>E.coli</th>
<th>Resources of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>5.6</td>
<td>44</td>
<td>24</td>
<td>390</td>
<td>8</td>
<td>0.0</td>
<td>0.0</td>
<td>Nil</td>
<td>RO</td>
</tr>
<tr>
<td>0.03</td>
<td>13.37</td>
<td>52.3</td>
<td>35.6</td>
<td>118</td>
<td>5.92</td>
<td>0.43</td>
<td>336</td>
<td>50</td>
<td>River water</td>
</tr>
</tbody>
</table>

Study design: The mice were assigned into three group (each group contain 6 females):

1--First group (control group) : It was given normal saline 0.9% NaCl.

2- Second group : It was given drinking good water (RO) for a two-month experimental period.

3-Third group : It was given river water for a two-month experimental period.

**Hematological assay**

After the end of experimental period, the mice were anaesthetized and blood was drawn directly from the heart by cardiac puncture. The hematological tests were done by using Genux Auto Hematology Analyzer where the results read and printed automatically.

**Statistical Analysis**

The data were analyzed by using Duncan test to determine mean and standard deviation, p≤0.05 was considered as significant in this study (7;8).

**Results and discussion**

The effect of drinking water quality on some hematological parameter (RBC, Hb, PCV and MCV)

The results showed a significant increase (p ≤0.05) in the count of red blood cells (RBCs) in the second and third group when compared with the control group and the results also showed a significant increase in the third group when compared with the second group.

The results also showed a significant decrease (p≤0.05) in the concentration of hemoglobin in the second group while it was significant increase in the third group compared with the control group, as is the case for achieving a significant increase in the third group when compared with the second group.

The results showed a significant decrease (p≤0.05) in the packed cell volume(PCV) in the second and third group compared with the control group. The results also showed a significant increase in the third group when
compared with the second group.

The results showed a non-significant decrease in mean corpuscular volume (MCV) in the second group and significant increase in third group compared with the control group. The results also showed a significant increase in the third group when compared with the second group as shown in table (2).

Table 2. The effect of drinking water quality on some hematological parameter (RBC, Hb, PCV and MCV), N = (6) (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Groups</th>
<th>(RBC×10^5/mm^3)</th>
<th>(Hb - g/dL)</th>
<th>(PCV%)</th>
<th>MCV (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First group</td>
<td>81.01 ± 1.1 a</td>
<td>12.8 ±1.3 a</td>
<td>44.6 ± 1.5 a</td>
<td>46.9 ±1.15 a</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second group</td>
<td>88.06 ± 2.82 b</td>
<td>11.9±.89 b</td>
<td>40.7 ±1.35 b</td>
<td>46.6 ±.74a</td>
</tr>
<tr>
<td>Third group</td>
<td>95.04 ± 1.23 c</td>
<td>14.5±1.17 c</td>
<td>44.2±.82</td>
<td>47.3 ±1.15 b</td>
</tr>
</tbody>
</table>

Differences of letters indicate differences of significant at( P≤0.05)

The reason for this increase in the count of red blood cells may be due to the influence of bacteria and parasites where these organisms affect the bone marrow, (especially in the reddening colonies) which is the site of the formation of red blood cells or the reason for this increase is the direct effect of these organisms on the cell divisions that generate red cells as they differentiate into erythrocytes.

Erythropoietin which is excreted from the kidneys as a result of a decrease in the percentage of oxygen supply to the body’s cells, controls the formation of erythrocytes in the bone marrow by stimulating the production of red blood cells and its role in preserving the red ancestors from programmed cell death and stimulating them to grow and divide until the formation of reticulocytes which develops into mature red blood cells. The secretion of this hormone weakens a lot when the kidneys get sick, as bacteria may secrete toxins that affect kidney. Therefore, the increase in the number of red blood cells may be due to the effect of bacteria on erythropoietin through two side, either affecting the site of its secretion or affecting the way it affects, while the first side may be the result of the effect of toxins of organisms on the kidneys where the site of the secretion of this hormone, and the other side of the effect on the mechanism of action of erythropoietin may be a result of the influence of germs on the sensitivity of the target cells to erythropoietin, thus losing its effectiveness and the degree of its effect in those cells.

The effect of drinking water quality on some hematological parameter (WBC, Lymphocytes, Monocytes, granular cells and PLT)

The results showed a significant increase (p≤0.05) in the count of white blood cells in the second and third group when compared with the control group and the results also showed a significant increase in the third group when compared with the second group.

The results showed a significant increase (p≤0.05) in the percentage of lymphocytes in the second and third group when compared with the control group and the results also showed a significant increase in the third group when compared with the second group.
The results showed a significant increase (p≤0.05) in the percentage of monocytes in the second group while it decreased significantly in the third group compared to the control group and the results also showed a significant decrease in the third group when compared with the second group.

The results showed a significant increase (p≤0.05) in the percentage of granular cells in the second and third groups when compared with the control group and the results also showed a significant decrease in the third group when compared with the second group.

The results showed a significant increase (p≤0.05) in the count of platelets in the second group while it decreased significantly in the third group compared with the control group and the results also showed a significant decrease in the third group when compared with the second group as shown in table (3).

Table 3. the effect of drinking water quality on some hematological parameter (WBC, Lymphocytes, Monocytes, Eosinophil and PLT), N = 6 (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC (×10³/mm³)</th>
<th>Lymphocyte %</th>
<th>Monocyte %</th>
<th>Eosinophil %</th>
<th>PLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>First group (control)</td>
<td>3.90±.56 a</td>
<td>55.60±1.08a</td>
<td>5±.12a</td>
<td>6.60±.54a</td>
<td>241±3.8 a</td>
</tr>
<tr>
<td>Second group</td>
<td>5.4±.53 b</td>
<td>77.29±.66b</td>
<td>13.15±.57b</td>
<td>9.40±.35b</td>
<td>690.2±7.91b</td>
</tr>
<tr>
<td>Third group</td>
<td>6.52±.41 c</td>
<td>89.01±.25c</td>
<td>2.6±.30c</td>
<td>8.57±.36c</td>
<td>229.49±1.06c</td>
</tr>
</tbody>
</table>

Differences of letters indicate differences of significant at (P≤0.05)

As for the increase of white blood cells in general and lymphocytes in particular in the blood of the second group, this increase may be a natural reaction to the threat represented by the entry of foreign bodies such as bacteria and parasites found in drinking water which was previously mentioned to prove their presence in such a type of water as it represents white cells are the main pillar of the immune system and the first to respond to combat any threat especially biological threats represented by this type of organism.

It is noted from the increase in the total count of white cells the difference of immune responses against such a type of threat, as the increase of lymphocytes becomes clear that there is a large activity in the production of antibodies by this type of cell or the increase in the numbers of killer lymphocytes while the increase in other cells, such as mono and neutral is Evidence of increased ingestion by this cell type against the bacterial species present in the water.

It has been observed from the results of the study that most of the increase is due to the count of lymphocytes and consequently an increase in their percentage compared to granular and monocytes which is observed to decrease in percentage (11).

The count of white blood cells may remain within the normal range or there may be an increase in parasitic infection (12).

(13) reported an increase in the count of WBC to more than 10,000 cells/mm³ in 11 patients out of 15 pinworm infections.

As for the reason for the decrease in the monocytes it may be due to the effect of toxins as some types of bacteria secrete a toxin called beta which analyzes the leukocytes, as well as the secretion of a toxin called kama, which causes the release of lysosome enzymes in the neutrophils. It leads to an increase in exudation by pumping the sodium element and thus an increase in
the flow of potassium ion. There are toxins that work to prevent the process of phagocytosis by phagocytosis, as well as remove the granules and consequently their angular death (10).

The results of the current study showed a decrease in the count of platelets in the blood of the third group when compared with the control group Platelets like other solid blood components result from the divisions, growth, and development of Megakaryocytes in the bone marrow under stimulation of the thrompoietin secreted by the liver which plays a major role in stimulating, multiplying and maturing those cells to form platelets (14) so low levels of thrombocytopenia may be caused by the presence of water-presenting organisms on the secretion of this hormone by decreasing its secretion as a result of the effect on the cells secreting it.

The results of the current study were in agreement with the results of a study (15) that showed an increase in the number of white blood cells while it was not consistent with them in the results of red blood cells which decreased in this study.

**Conclusion**

We conclude from this study that the water source and its quality have a significant effect on the blood parameters of laboratory animals, and then influence on the animal’s health.

**Financial Disclosure**: There is no financial disclosure

**Conflict of Interest**: None to declare

**Ethical Clearance**: all experimental protocol were approved under university of Thi-Qar collage of education for pure sciences , biology department and all experimental carried out accordance with approved guidelines.

**Reference**


7- Duncan, D. B. Multiple range and multiple F tests. Biometrics1955; 11(1), 1-42.


Assessment of Nurses’ Knowledge Concerning Early Detection for Hypothyroidism/Hyperthyroidism in Baghdad Teaching Hospitals

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²Prof, Fundamentals of Nursing Department, College of Nursing-University of Baghdad

Abstract

Hypothyroidism and Hyperthyroidism is the most common endocrine disorders in clinical practice. Critical illness is often associated with dysfunctions of thyroid hormone. Thyroid dysfunction is a serious matter if managed inaccurately; it early detection may decrease morbidity and mortality. **Objectives**: Assessment of nurses’ knowledge concerning early detection for hypothyroidism/hyperthyroidism in Baghdad Teaching Hospitals and to find out a relationship between nurses knowledge with some variables such as (age, gender, educational level, and marital statues). **Methodology**: A descriptive study was conducted in the period of December 20th 2019 up to the end of May 28th 2020. The sample consisted of (50) nurses who were systematically selected one by one. The data collected was analyzed using SPSS version 22.0. **Results**: The majority of the study were female who accounted for (72%) of the total participants while male constituted (28%). Most of the study participants (34%) were ages 26-30 years old. (44%) of the nurses were diploma graduate. Fifty percent of the nurses were married. More than half (58%) of nurses had experience years in nursing and (80%) of nurses had training course in hospitals, (66%) have number of training were 1-5 course and (80%) had location of courses in Iraq. **Conclusions**: Findings of study demonstrate that, nurses have good knowledge about early detection of Hypothyroidism/Hyperthyroidism. **Keywords**: Knowledge, Early detection, Nurses, Hypothyroidism, Hyperthyroidism

Introduction

The thyroid gland is regulated by the thyroid-stimulating hormone (TSH) which is secreted by the anterior pituitary and it’s very important for metabolism of the body through secretion of two hormones, thyroxine (T4) and triiodothyronine (T3) [1]. Hypothyroidism, which has affecting of 2% - 5%, is the most common form of thyroid dysfunction [2]. Hypothyroidism and hyperthyroidism are correct etiological, anatomical and functional diagnosis of the thyroid problem can be achieved by take history of patient, thorough physical examination include weight loss/weight gain, palpitation, change of bowel habits (diarrhea/constipation), sweating, sleep problems, menstrual irregularities, growth problems, delays in sexual maturation, infertility, hoarseness of voice, exophthalmos, tremors, atrial fibrillation and thyroid gland enlargement and by well-planned investigations that include serum T₃,T₄, and TSH [3]. Currently, neonatal early detection elevated levels of TSH that increase in response to the reduction in thyroid hormone. This early detection identifies 90% of cases of congenital hypothyroidism. Most patients have normal development after treatment with thyroxin. Besides assay of TSH, triiodothyronine (T3) and thyroxin (T4), other diagnostic tests include thyroid scanning with radioactive iodine, thyroid echography, and assay of serum thyroglobulin. These exams can help determine the causes of the disease and differentiate permanent and transient cases [4]. Early detection of thyroid disorder depended on physical examination and laboratory test of thyroid hormone. Differential diagnosis is based on absence of exophthalmos and presence of myxedema, anti-TSH antibodies and lymphocyte infiltration of the thyroid gland [5]. Undiagnosed thyroid disorder may put patients at higher risk for certain life threatening conditions such as cardiovascular disease, osteoporosis and infertility [6].

DOI Number: 10.37506/ijfmt.v15i1.13655
Materials and Methods

A descriptive study: Assessment of nurses’ knowledge concerning early detection for hypothyroidism/hyperthyroidism in Baghdad Teaching Hospitals. This study was conducted at Baghdad Teaching Hospitals between December 20th 2019 up to the end of 28th May 2020. A tool of knowledge questionnaire was developed and distributed to the participants in this study. The sample consisted of (50) nurses at Baghdad Teaching Hospital, AL.Kindy, AL.Yarmouk Teaching Hospital, Imamein Kadhimein Medical City. A questionnaire-interview format was designed and developed by the researcher for the purpose of the study; such development was employed through the available literature, clinical background and interview with nurses. All the items were measured on scale of (2) indicates that the know 2; don’t know 1. The questionnaire consisted of (2) parts. Part I: Demographic Information Sheet. Part II: Assessment of Nurses’ Knowledge Concerning Early Detection for Hypothyroidism/Hyperthyroidism. Rating scale was used to rate the frequency and extension of the problems. The content validity of the instrument was established through a panel of (14) experts. Test-retest reliability was determined through a computation of person correlations for the scales. The data were collected by using the questionnaire structured format through interview and inspection technique. The determination was conducted during the period from 15th January 2020 to 30th February 2020. The data were analyzed through descriptive data analysis and inferential data analysis the data were analyzed through the use of Statistical Package of Social Sciences (SPSS) version (22).

Results

Table (1): Descriptive Analysis of Nurses’ knowledge toward early detection of Hypothyroidism/Hyperthyroidism Items.

<table>
<thead>
<tr>
<th>Items</th>
<th>frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>2</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Fair</td>
<td>23</td>
<td>46.0</td>
<td>46.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Good</td>
<td>25</td>
<td>50.0</td>
<td>50.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

This table shows that high percentage (50.0%) were good knowledge, (46.0%) had fair knowledge and only (4.0%) poor knowledge toward early detection Hypothyroidism/Hyperthyroidism.

Table (2): Association between (age, gender Level of Education, and martial statues) and Nurses Knowledge.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>8.6628</td>
<td>2</td>
<td>4.331</td>
<td>2.128</td>
<td>.130</td>
</tr>
<tr>
<td>Within Groups</td>
<td>95.658</td>
<td>47</td>
<td>2.035</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Total</td>
<td>104.320</td>
<td>49</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.0816</td>
<td>2</td>
<td>.0409</td>
<td>.189</td>
<td>.828</td>
</tr>
<tr>
<td>Within Groups</td>
<td>9.9999</td>
<td>47</td>
<td>.2133</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Total</td>
<td>10.0809</td>
<td>49</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>
Cont.. Table (2): Association between (age, gender Level of Education, and martial statues) and Nurses Knowledge.

<table>
<thead>
<tr>
<th></th>
<th>Between Groups</th>
<th>.267*</th>
<th>2#</th>
<th>.134#</th>
<th>.264#</th>
<th>.769#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td>Within Groups</td>
<td>23.813#</td>
<td>47#</td>
<td>.507#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24.080#</td>
<td>49#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Martial statues</td>
<td>Between Groups</td>
<td>2.165#</td>
<td>2#</td>
<td>1.083#</td>
<td>2.084#</td>
<td>.136#</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>24.415#</td>
<td>47#</td>
<td>.519#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26.580#</td>
<td>49#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

*: P ≤ 0.05; *: P ≤ 0.01**

This table indicates that there is no significant association between age, gender, level of education, and martial statues with nurse’s knowledge.

**Discussion**

Through the course of the data analysis of the present study the finding showed that the majority (72%) of the study were female while the remaining was male. The highest percentage of age group in present study (34%) were 26-30 years old and lowest percentage(10%) were (31- 35,36-40) years old. Concerning marital status and level of education, (50%) of the sample were married, most of them are (44%) nursing had diploma graduate.

These results are agreement with the findings obtained from other study, who shows 69% of the study sample were females within age group that (23-27) years old from Iraqi Center, high percent of the three hospitals were married and divorced, (40%) of the sample nursing had diploma graduate[6].

Regarding experience years, majority (58%) of the study was experience years in nursing and (80%) of nurses had training course in hospitals, (66%) have number of training were 1-5 course and (80%) had location of courses in Iraq. These results are accordance with findings obtained from other study, shows that after the training course, high percent of nurses had (1 - 5) years of experiences in hospital,(73.3%) of nurses have theoretical training course in Iraq[7]. This study similarity with other researcher show that number of years in the health field, the majority of both groups of nurses have (1-5 years) of experience years in nursing field[8]. The result of accurate study that show early detection of hypothyroidism/hyperthyroidism domain in table (1) for nurses knowledge is fifty percentage were good knowledge, forty six percentage had fair knowledge and only four percentage poor knowledge toward early detection Hypothyroidism/Hyperthyroidism. A finding suggest the large number of patients to enter to medical word ,critical care unit ,and surgical word that suffering singes and symptom of thyroid disorder leads to frequent nurses cope with these problem to provide nursing care and to increase knowledge about these health problem concerning hypothyroidism and hyperthyroidism. The findings of the study disagree with result obtained from other study who reported that the most of the nurses had a fair level of knowledge (54%) and the lowest percentage (12%) of nurses’ knowledge in good knowledge toward hypothyroidism and hyperthyroidism. This finding difference to a study conducted by other researcher who stated that the not acceptable of nurses’ knowledge (43.3%), acceptable level (23.3%), fail level (25.0%), and no excellent level toward thyroid disorder management[10]. This finding are good agreement done which other researchers showed that the study finding total post knowledge means of score was higher than total pre knowledge mean of score[11]. Level of education ,experience of nursing in hospitals in comparison of the respondent’s total Nurses knowledge domain in table (2) ,there is non-significant association between age ,gender ,level of education , and
martail statues with nurses knowledge domains. This result of study are disagreements with other studies done by other researchers whose reported that the association between knowledge and education level shows there was high significant relationship between nurses level of knowledge and age of nurses at P = P ≤ 0.05 level [7]. These result were similar to those result obtained from other researcher who reported that no significant difference between study and control groups related to age group ,gender, level of educational, current work place with knowledge domain (P<0.0001) [8]. Other study show that no statistical significant relationships between nurses’ gender and nurse’s knowledge [9].

**Conclusions**

This study demonstrates that, despite good nurses’ knowledge regarding early detection of hypothyroidism and hyperthyroidism.

**Recommendations:**

1. The study suggested opening endocrine centre in Baghdad city to early detection hypothyroidism and hyperthyroidism.

2. Increases training course for nurses outside in Iraq that contributed to enhance nurses’ knowledge and practice about early detection hypothyroidism and hyperthyroidism.

This research was funded by Author. Moreover, we would like to thank the study participants and data collectors for their fully participation and responsible data collection.

**Funding:** None

**Conflict of Interest:** None declared

**Ethical approval:** Not required

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Assessment of Employees’ Knowledge Concerning Contributing Factors and Early Detection for Prostate Cancer in Baghdad University Colleges in bab-Almudam

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Abstract

Worldwide prostate cancer is the second most frequent cause of cancer deaths. Screening techniques is useful for improving survival rates and treatment outcomes can be employed to detect the disease earlier in apparently healthy individuals, and increasing evidence shows that this can decrease morbidity and mortality of the disease.

Objectives: The objectives of this study was to assess the knowledge concerning contributing factors and early detection to screen for prostate cancer among male staff of the University of Baghdad Colleges in bab-Almudam Area, Iraq and to find out the relationship between employees’ knowledge and their demographical characteristic.

Methodology: This cross-sectional descriptive design study was carried out with 100 male staff working in colleges of Baghdad University in bab-Almudam region. Male staff who agreed to participate and were recruited on giving oral consent. Knowledge about prostate cancer and screening was operationalized through 28 items, including 12 items from the Knowledge about Prostate Cancer Screening Questionnaire, and 16 items assessing contributing factors and prostate cancer screening controversy. Data was collected by distributing structured a self-administered questionnaire, written in Arabic was used.

Results: The Majority of participants (36.0%) were ages between (38 -47) years, the median age was 36 years (range, 18-36) years (mean=41.8+ S.td =10.035). Regarding marital status, the majority (77 %) was married, followed by (19%) who were single and (4%) who were divorced or widowed. One third (38%) of participants had PhD education, following by (26%) secondary school, (18 %) university,(9%) intermediate, (5%) MSc and (4%) institute education. Furthermore, (89%) of study participants reported they had no family history of prostate only (11%) reported had family history PCa. A total of (94%) were living in urban area and reminder lived in rural area. About (48%, 35%) respectively, of respondents had a medium and high knowledge level about prostate cancer and early detection screening methods.

Conclusions: This study indicated that the staffs of University of Baghdad Colleges have appreciable knowledge regard contributing factors and early detection to prostate cancer screening. A significant proportion of staff however, exhibited poor knowledge of prostate cancer screening and contributing factors to prostate cancer.

Keywords: Assessment, Employees’, Knowledge, Contributing Factors, Early Detection, Prostate Cancer

Introduction

Prostate cancer is neoplasm of the male prostate gland and is one of the most common cancers in the world wide and it is the second leading cause of cancer related
deaths among men globally. The symptoms of prostate cancer may develop slowly, but some early signs include difficult and frequent urination, erectile dysfunction, pain in lower back, pain in lower pelvic area, and blood in urine. Uncontrolled risk of developing the disease such as age, race and family history are associated to prostate cancer, however PCa awareness and early detection may positively affect the life of adult male, and improve their life style (1). The American Cancer Society (ACS) recommend the prostate specific antigen test (PSA), and digital rectal (DRE) examination, as the common screening modalities for Prostate cancer for men at high risk such as a family history, racial differences regarding the lack of access to health services, and socioeconomic conditions should performing prostate-specific antigen (PSA) testing, also recommends that men older than 50 years old, should be informed about early detection of prostate cancer screenings. They should be advised at an earlier age about this risk. An early detection through screening and timely treatment provides the greatest chance for increasing the 5-year survival rate. If the cancer is diagnosed during the early stages, the PCa prognosis is more optimistic (1,2,3).

**Methodology**

A cross-sectional descriptive design study carried out in the four Colleges of University of Baghdad to determine employees’ knowledge concerning contributing factors and early detection for prostate cancer among employees in Baghdad University Colleges. The participants of this study were conveniently sampled from the four faculties of the University of Baghdad. Male staff who accepted to participate gave oral consent and was recruited. A total of (100) male staff participated in the study. Inclusion criteria for participants were a) employees at the age of 18 – 63 years old; b) those who communication was possible, able to read and write and no suffering from any psychiatric disorders) those who understood the purpose of research and allowed for such participation whereas the exclusion criteria were males who were then diagnosed as benign prostate hyperplasia, prostate cancer or were being treated for prostate cancer. The questionnaires were including the Knowledge about contributing factors and early detection methods screening condition on prostate cancer. Knowledge of prostate cancer questionnaire: a draft of the 28-item based on literature review and previous research related prostate cancer by researcher. Thereafter, the validity of the questionnaire was verified by 5 experts’ specialist in urology, 13 experts in adult nursing department with more than 10 years of experience. As a result of the tool’s test-retest reliability was $r = 0.84$. Each correct answer was scored two to a correct answer while an incorrect answer scored to one-point. Firstly, we conducted a questionnaire preliminary survey to 10 employees over 18 years old and then modified difficult or understood terms into easy ones in a questionnaire. Data was collected from 5$^{th}$ December 2019 to 3$^{rd}$ March, 2020. The data was collected through distribution of self-administered questionnaire. The instrument comprised 32 questions, which included (6) questions related participants demographic characteristics and (28) questions related contributing factors and early detection methods for prostate cancer. The collected data was analyzed using SPSS statistical package (version 22.0). Descriptive analyses were used to describe the characteristics of the participants related to prostate cancer knowledge: frequency, percentage (%), mean and standard deviation. one-way ANOVA were used to explore difference of knowledge of prostate cancer according to characteristics of the employees and prostate cancer at p < 0.05 was considered statistically significant for all test.

**Results**

Participants age range was from (38 to 47) years-old with an average age of 36 years (mean=41.8+ SD =10.035) for the study participants. Most participants had doctorate education (38%), (26%) secondary school, (18 %) university,(9%) intermediate , )5% MSc and (4%) institute education respectively and about (77%) of respondents indicated that they were married and, (11%) of them were single and reminder (4%) were divorced or widowed. The majority of the respondents (89%) indicated having no family history of prostate cancer and (11% ) had no family history of prostate cancer. Participants reported residing majority in urban (94%) areas. The mean and standard deviation of the knowledge was 43.60 ± 5.312, most of them 48% of the participants having a moderate level of knowledge about contributing factors and early detection tests of PCa.
Table 1: Descriptive Analysis Level of Participant’s Knowledge concerning Contributing Factors and Early Detection for Prostate Cancer

<table>
<thead>
<tr>
<th>Items</th>
<th>frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>17</td>
<td>17.0</td>
<td>17.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Medium</td>
<td>48</td>
<td>48.0</td>
<td>48.0</td>
<td>65.0</td>
</tr>
<tr>
<td>High</td>
<td>35</td>
<td>35.0</td>
<td>35.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Association between Employees’ Knowledge and Socio-Demographic Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sum of Squares</th>
<th>D.F</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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</thead>
<tbody>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Between Groups</td>
<td>7.163</td>
<td>2</td>
<td>3.582</td>
<td>3.768</td>
<td>.027</td>
</tr>
<tr>
<td>Within Groups</td>
<td>92.197</td>
<td>97</td>
<td>.950</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99.360</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.371</td>
<td>2</td>
<td>.186</td>
<td>.761</td>
<td>.470</td>
</tr>
<tr>
<td>Within Groups</td>
<td>23.669</td>
<td>97</td>
<td>.244</td>
<td></td>
<td></td>
</tr>
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<td>Total</td>
<td>24.040</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>50.082</td>
<td>2</td>
<td>25.041</td>
<td>7.670</td>
<td>.001</td>
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<tr>
<td>Within Groups</td>
<td>316.668</td>
<td>97</td>
<td>3.265</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>366.750</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family history of PCa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.761</td>
<td>2</td>
<td>.380</td>
<td>4.086</td>
<td>.020</td>
</tr>
<tr>
<td>Within Groups</td>
<td>9.029</td>
<td>97</td>
<td>.093</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9.790</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.091</td>
<td>2</td>
<td>.046</td>
<td>.799</td>
<td>.453</td>
</tr>
<tr>
<td>Within Groups</td>
<td>5.549</td>
<td>97</td>
<td>.057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.640</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Throughout the course of the data analysis of the current study, the findings show the majority of the participants (36%) age groups ranged from 38-47 years, the average age was 36 years (mean=41.8, S.td =10.035). On marital status majority of employees (89%) were married. Most of respondents (38%) had doctorate education, (26%) secondary school, (18 %) university,(9%) intermediate , (5%) master and (4%) institute holder education, respectively. Majority of them (89%) have no family history of PCa. Finally high percentage of employees (94%) was living in urban area. These findings agreed with study done by Awosan, who reported the ages of the respondents ranged from 40 to 84 years (Mean = 53.13 ± 7.92) with a larger proportion (44.7%) in the 40-49 years age group. Most of the respondents were married (84.0%). Majority of respondents (55.4%) had little education (4). These findings are similar to Abuadas who reported the mean age of participants was 52.5 years (SD=8.5; range=40-75). A total of 42.1% had a primary educational level, 29.9% had a secondary educational level, and 28% had a university education. Regarding marital status, the majority (91.4%) was married, followed by 5.6% who were single and 3% who were divorced or widowed (5). The ages of the respondents ranged from 45-60. The mean age of the study respondents was 49.52 (SD=± 3.95), with the majority of them, 68.1%, in the age category 45-50 years. Most of the respondents, 87.5%, indicated that they were married and 12.5% were single. 90.6%, of the respondents indicated they had completed University education while 9.4% had a 3- year post-secondary education (6).

Twenty eight questions assessed general knowledge of employees concerning contributing factors and early detection methods. Most, 48 (48%) of the 100 participants had fair knowledge of prostate cancer, while 35 (35%) of them had good and only (17%) had poor knowledge about prostate cancer. The finding revealed that participants have scored 80% and above in only (8) questions. They scored between 50% and 79% in (13) questions and they scored less than 50% in seven questions. The highest score was 88%. These findings are similar to Kaninjing as he found A majority of participants (55.2%) exhibited medium knowledge of prostate cancer. In terms of awareness of the PSA test, 88.8% of participants were not aware of this screening method while 91.0% were also not aware of the DRE (7). The study was also supported by study done in Malaysia by Firzara, et al., who found a majority of the participants correctly answered the questions on ‘increased of age more than 50 years’ (97.4%) and increase the risk of prostate cancer ‘with person having a first-degree relative of PCa (82.7%). Only (31.1%) of respondents were aware the risk increases with individuals who having a first-degree relative of prostate cancer (8). This finding was in good agreement with that obtained by Morland who stated that the majority of men had an adequate knowledge about prostate cancer (82.1%) (9). A similar findings are accordance with another study carried out in Jamaica, with 96% of participants answered correctly to questions concerning prostate cancer (10). This finding is the same line with study done by Adibe that revealed the greater majority of male staff who demonstrated a high level of knowledge of prostate cancer had tertiary degree (94.9%, n = 356.0). Academic staff constituted the greater majority (77.1%) of staff with high knowledge level of prostate cancer (11).The association between socio-demographic and employees’ knowledge score was explored. There are significant association between ages, level of education, family history of prostate cancer and studied sample knowledge at p value ≤ 0.05 and also illustrate that no relationship found with rest of studied variables. These findings are disagreeing with findings obtained from Yeboah. Fisher’s exact test statistics revealed that there was no association between age, marital status, level of educational, current rank, family history of PC and knowledge about prostate cancer (12,13) . These findings good agreements with many studies study, those reported that the association between social –demographic characteristics’ and prostate cancer knowledge among the participants; All demographic data were significant associated with knowledge of prostate cancer except social status (there is no significant associated with knowledge about prostate cancer (p=0.337), While chi-square indicated there are significant association between age, marital status, and the prostate cancer knowledge (14, 15, 16, 17) .

Conclusions

In conclusion, the results of the current study show that the level of knowledge among the employees’ about prostate cancer is enough in general, but the
participants in the study demonstrated low knowledge in early detection of prostate cancer and early signs and symptoms. The study found the association between sociodemographic variables and knowledge of prostate cancer, except the marital status and residence which are not associated to the knowledge of prostate cancer. The study recommended the Ministry of health (MOH) should organize public health campaigns using the mass media, social media such as Facebook, twitter and hospital to improve knowledge, attitude and use of screening practices related to early detection methods of prostate cancer. Also we recommended establishing of educational program to improve knowledge and attitudes related contributing factors of and early detection of prostate cancer for university of Baghdad employees.

Acknowledgements: This research was funded by Authors. Moreover, we would like to thank the study participants and data collectors for their fully participation and responsible data collection.

Conflict of Interest: None declared.

Ethical approval: The study was approved by the Institutional Ethics Committee.

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Abstract

Introduction: In healthy population, temporal parameters of gait like step time and stride time are frequently linked with gait cycle in healthy populations but can be obstructive, difficult and time taking to measure. This study is to check the efficacy of kinect azure for evaluating temporal parameters of gait in normal healthy people. A kinect based recording application was started & use to detect , tracks human post analytical motions. Kinect is low cost, unobstructive and accurate gait analysis application with various uses like monitoring, diagnosis, rehabilitation & management. The end result of this study is to fed a state system that analysis the present state from which the assessment are withdrawn.

Method: The research has been structured as an observational study. The total of 132 participants will be taken from AVBRH, sawangi Meghe for study as per inclusion and exclusion criteria. With intervention the period of study will be 6 months. It holds single period, concurrent validity evaluation comparing temporal gait parameters derived from the Kinect system.

Discussion: This study protocol aims to evaluate the Validity of evaluation of temporal parameters of gait using Microsoft Kinect Azure. The study’s expected outcome will concert on the evaluation of temporal parameters of gait using Microsoft Kinect Azure in normal healthy population.

Keywords: Kinect azure, temporal gait analysis, healthy individuals and projector.

Introduction

Research has shown the importance of measuring a person’s gait and that the parameters describing locomotion. Recovery of functional & independent ambulation is big rehabilitation goal. A complete gait analysis determine deviations & damage underlying decreased functions & so, this may assist in decision making as well as in evaluating rehabilitation usefulness . Gait analysis can be a effective tool to differentiate among disease structure & to determine health & danger of disease and injury like fall detection and prognosis among the elderly individual. Gait impairments prevail in abundant clinical populations and the senior citizen.

Recent studies have shown that the number of stride-to-stride variation in the calculation of stride length, velocity & speed are individual predictors of future falls ,thus can be handy to recognize high risk citizen. Wearable sensors are proposed under recent studies. Such devices are lightweight, small, less expensive & portable (Figure:2). Despite of there superiorties, wearable sensors have few disadvantage. Sensors should be placed securely and precisely, sensors are obstructive in a way that it needs daily routine changes for subject. It also requires service of charging battery, uplinking data & sanitary treatment. Accurate, unobstructive, low cost gait analysis structure have many uses like monitoring,
Recent evidence shows that the Kinect, which utilize depth and image sensor data merge with AI algorithms to recognize anatomical landmarks without need of sensors attached to the individual’s body (Figure:1). Further, research reveals that the Kinect is able to validly evaluate stride dynamics in walking. Present devices that have ability to precisely measuring spatiotemporal & kinematic gait variables are costly, prolong and less handy. Clinicians have lately used commercially accessible gait measurement device to evaluate the temporal & spatial parameters of the footstep pattern. Variables that can be evaluate include walking speed, single and double limb support duration, and stride width, cadence for steps and Step sequence.

The Microsoft Kinect azure is a cheap gaming device that has shown assurance as a clinical assessment tool. Thus, the objective of this study was to assess the validity and reliability of kinect when assessing spatiotemporal parameters of gait. The major limitation for the Kinect azure is that it keep good data quality only in the scale of few meters from the sensor and keep on degrading when goes further. Thus, the purpose of the study was to evaluate the validity and efficiency of kinect when evaluating spatiotemporal and gait parameters.

**AIM AND OBJECTIVE:**

**Aim:**
To assess the validity of stride time & step time in normal population.

**Objective:**
1) To evaluate stride time in normal individuals by Microsoft kinect azure.
2) To evaluate step time in normal individual by Microsoft kinect azure.

**Methodology**
This study will be managed in the Department of Community Health Physiotherapy at Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, India, with the approval of Datta Meghe Institute of Medical Sciences, Institutional Ethics Committee and Deemed to be University.

**Study setting:** Ravi Nair Physiotherapy College

**study type:** observational type

**sampling technique:** simple random

**sample size:** 132 participants

**study duration:** 6 months

**Material Required:**
1) Consent form
2) Projector
3) Microsoft kinect azure
**Procedure:**

We were collecting images of 132 subjects (both male and female). Subjects are instructed to walk on straight line. Sensor is placed to record the image of the subject. The Kinect sensor is mounted at each experiment to capture the subject's image. The distances are compatible with the recommendation for achieving the highest data quality. This distance enables to record 1 full gait cycle (i.e., complete stride) per walking trial for each limb that does not include the initial step and final stage of the pathway.

The Kinect-based recording structure is developed and used for follow identification & capture the human pose & post analytical motion. The temporal gait parameters were extracted by Kinect: step time (both foot), step length (both foot), stance time (both foot), and velocity. If one limb is in swing phase the other foot is in single limb support phase than limb goes through a double limb support phase during stance phase. A single limb support phase (when the other limb is off the ground), which means another double limb support phase. Fig. 3 shows the flow chart of the study.

**FIGURE 3: flow chart of the study**

**OUTCOME MEASURE:**
Temporal parameter of gait.

**Participant selection:**

Exclusion criteria:

1) Individual with abnormal gait.
2) Lower limb fracture.
3) Traumatic injuries of lower limb.
4) Neurological problem.

Inclusion criteria:

1) Normal individual without gait impairment.
2) Subjects willing to participate voluntarily.
3) Both male and female patients.

**Discussion**

The purpose of the study protocol is to evaluate gait parameters with Microsoft Azure in normal individuals. Its expected outcome is based on the temporal gait parameter evaluation using Kinect Azure. This research helps to assess the efficacy of Kinect Azure in the assessment of gait parameters in healthy population.

**Result**

The study’s expected outcome will focus on evaluation of temporal parameter of gait in healthy individuals. After accomplishment of study result will be calculated by systemic data analysis by randomized control trial.

**ETHICS AND DISSEMINATION:**

The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review.

**Source of Funding:** There will be no direct support for this research from public and private organizations. The department of physiotherapy, at Datta Meghe Institute of Medical Science, Deemed to be University will provide material needed for research.

**Conflict of Interest:** Nil

**References**


Morphometry and Morphology of Acromian Process of Scapula

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Abstract

Introduction: The acromian process of scapula is project forward from lateral end of spinous process of scapula. Coracoacromial ligament is extend between coracoid process and acromian process, it help in formation of coracoacromial arch, anatomy of this region is important for better understanding of pathology regarding subacromial bursa, impingement syndrome.

Materials and Methods: Present study was carried out on 74 dry, adult human scapulae 38 left side and 36 right side from teaching collection of anatomy department, sex of the specimen not known, Scapular length, width, acromian process length, width, thickness, coracoacromial distance, acromioglenoid distance was measured with the help of vernier caliper, we also found different shape of acromian process.

Results: We observed 74 scapula out of them 38 scapula are of type –I which was 51.35%, 13 scapula of type –II which was 17.57%, 23 scapula of type –III which was 31.08%, we observed mean value acromian length was 4.50±0.52cm, width of acromian process was 2.10±0.26cm, thickness of acromian process was 0.78±0.10cm, acromicoracoid distance was 3.45±0.53cm, distance between acromian process and supraglenoid tubercle was 2.48±0.40cm.

Conclusion: These findings suggest that the difference in various parameters related to acromian process help in better understanding of anatomy and pathology of coracoacromial arch, rotator cuff and also helpful to other clinician.

Key Words: Scapula, Acromian process,

Introduction

The acromian process projects forwards and continue from the lateral end of the spinous process of scapula on posterior aspect. In Spinous (process) projection lower border of the scapula becomes extend as lateral (posterior) border of the acromian process, this point is known as acromian angle that form a significant surface marking point.

The upper border of spine of scapula continue as medial border of acromian process, on acromian process having facet for with lateral end of clavicle bone form plane synovial joint acromioclaviclar joint. subacromial bursa located below the acromian process it separates acromian process and deltoid from supraspiantous tendon. coracoacromial arch formed coracoids process, coracoacromial ligament, anterior part of acromian process. Any pathology whether it is acquired or congenital, in this area reduce the area of this space can cause mechanical impingement.
This arch is fairly a non-elastic structure and it comprises of a subacromial space that is 1-1.5 cm wide and has the subacromial bursa, strong rotator cuff, and long head of biceps brachi muscle tendon. [2]

The anatomy of the acromian process, coracoacromial arch, coracoid process and other anatomical structures in this area is of clinical importance, to carry out analysis of scan, carrying out physiotherapy, and surgical aspect related with this shoulder joint. [3]

Knowledge of Acromian process anatomy provide better understanding in infringement syndrome and the pathology of rotator cuff. [4]

**Aim**

The aim of the current study was to measure the various parameters of acromian process of the scapula and to analyze the morphological aspect of the acromian process for better understanding and management of shoulder pathology.

**Material and Methods**

The present study was performed in Anatomy Department in Smt. B.K. Shah medical institute & Research center, Sumandeep Vidyapeeth university. A total of 74 dry scapula (36 right side and 38 left side) bones were deliberated from teaching collection of the Department of Anatomy. The bones are of belongs to adults and sex and age of the scapula were not recognized. Entirely scapula chosen were dry, showed normal anatomical features and deformed bones were excluded from study. All measurement was carried out by the digital vernier calliper. The data were then entered and scrutinize using the Microsoft software.

The following parameters of the acromian process were studied. All the measurements were taken manually by using of a digital vernier caliper and recorded in centimetres (cm)

- Acromion projection Length (AP diameter) - Distance along the longaxis from anterior to posterior
- Acromion projection Width (transverse diameter) - Distance between lateral and medial borders (maximum far points on this border)
- The coraco-acromial ligament was measured by using method of Edelson and Taitz [5]
- Coraco acromian distance (C-A distance) - Distance between acromian processes and tips of coracoid.
- Acromian thickness (breadth) - Thickness of anterior aspect was recorded at a point 1 cm lateral to medial border and 1 cm medial to lateral border.
- Acromio-glenoid distance (A-G distance) – Distance between two point glenoid tubercle superior aspect and inferior surface of acromian process.

**Result**

In present study we have taken 74 dry scapulae (36 right side and 38 left side) of unknown sex and age without any gross pathology, from teaching collection from anatomy department, we observed all 3 types of scapula. (table-1)

<table>
<thead>
<tr>
<th>Type</th>
<th>Right (n=36)</th>
<th>Left (n=38)</th>
<th>Total (n=74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1(Flat)</td>
<td>20(55.56%)</td>
<td>18(47.37%)</td>
<td>38(51.35%)</td>
</tr>
<tr>
<td>Type 2(Curved)</td>
<td>06(16.67%)</td>
<td>07(18.42%)</td>
<td>13(17.57%)</td>
</tr>
<tr>
<td>Type 3(Hooked)</td>
<td>10(27.78%)</td>
<td>13(34.21%)</td>
<td>23(31.08%)</td>
</tr>
</tbody>
</table>

On right side, we observed 20 out of 36 scapula of type –I which was 55.56%, 06 out of 36 scapula of type –II which was 16.67%, 10 out of 36 scapula of type –III which was 27.78% (Table-1)
On left side, we observed 18 out of 38 scapula of type –I which was 47.37%, 7 out of 38 scapula of type –II which was 18.42%, 13 out of 38 scapula of type –III which was 34.21% (Table-1).

We observed 74 scapula out of them 38 scapula are of type –I which was 51.35%, 13 scapula of type –II which was 17.57%, 23 scapula of type –III which was 31.08% (Table-1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total mean (cm)</th>
<th>Right side (cm)</th>
<th>Left side (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scapular.length</td>
<td>14.31±1.17</td>
<td>14.21±1.22</td>
<td>14.53±1.13</td>
</tr>
<tr>
<td>Scapular.Width</td>
<td>10.30±0.76</td>
<td>10.10±0.77</td>
<td>10.15±0.75</td>
</tr>
</tbody>
</table>

**Table: 2 Mean value of scapular measurements**

We observed mean value of scapular length on right side was 14.21±1.22 cm, mean value of scapular length on left side was 14.53±1.13 cm, mean value of scapular width on right side was 10.10±0.77 cm, mean value of scapular length on left side was 10.15±0.75 cm. (Table-2)

**Table: 3 Mean value of various parameters of Acromian process**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>mean (cm)</th>
<th>Right side (cm)</th>
<th>Left side (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of Acromian (AP diameter)</td>
<td>4.50±0.52 cm</td>
<td>4.55±0.52 cm</td>
<td>4.48±0.53 cm</td>
</tr>
<tr>
<td>.Width of Acromian (Transverse diameter)</td>
<td>2.10±0.26 cm</td>
<td>2.05±0.27 cm</td>
<td>2.15±0.24 cm</td>
</tr>
<tr>
<td>Thickness of Acromian (breadth)</td>
<td>0.78±0.10 cm</td>
<td>0.73±0.11 cm</td>
<td>0.80±0.08 cm</td>
</tr>
<tr>
<td>Coraco acromian (AC) Distance (interval)</td>
<td>3.45±0.53 cm</td>
<td>3.41±0.55 cm</td>
<td>3.49±0.52 cm</td>
</tr>
<tr>
<td>Acromo-glenoid Distance (interval)</td>
<td>2.48±0.40 cm</td>
<td>2.45±0.44 cm</td>
<td>2.52±0.36 cm</td>
</tr>
</tbody>
</table>

**Table -4 Comparision of types of scapula with previous studies**

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>32</td>
<td>10</td>
<td>22.5</td>
<td>13.3</td>
<td>28</td>
<td>37</td>
<td>5.20</td>
<td>51.35</td>
</tr>
<tr>
<td>II</td>
<td>22</td>
<td>73</td>
<td>38.8</td>
<td>81.88</td>
<td>67</td>
<td>48.7</td>
<td>57.9</td>
<td>17.57</td>
</tr>
<tr>
<td>II</td>
<td>46</td>
<td>17</td>
<td>38.8</td>
<td>4.72</td>
<td>05</td>
<td>13.7</td>
<td>36.9</td>
<td>31.08</td>
</tr>
</tbody>
</table>
In our study we have measured various parameters, found various shape and compare with other studies we have found 38(51.35%) out of 74 scapula of type I scapula, this value is higher than what observed in study of chandni gupta et al[6] found, singh at al[7], musa et al[10] 32%,22.5%, 37% respectively. Coskun at al[3], shilpi gosavi et al[8] reported type I scapula 10%,13.3% respectively. (Table-4)

We have found 17.57% scapula of type II scapula, this value is lower than what observed in study of chandni gupta et al[6] found, singh at al[7], musa et al[10] 22%,38.8%, 48.7% respectively. Coskun at al[3], shilpi gosavi et al[8] reported type II scapula 73%,81.88% respectively. (Table-4)

We have found 31.08% scapula of type III scapula, this value is lower than what observed in study of chandni gupta et al[6] found, singh at al[7], Schetino et al[11] 46%,38.8%, 36.9% respectively. Coskun at al[3], Shilpi gosavi et al[8] reported type III scapula 17%,4.72% respectively. (Table-4)

<table>
<thead>
<tr>
<th>Table -5 Comparision of various parameters of scapula with previous studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement Of Length (AP diameter)</td>
</tr>
<tr>
<td>Measurement Of Width (transverse diameter)</td>
</tr>
<tr>
<td>Measurement Of Thickness (breadth)</td>
</tr>
<tr>
<td>A-C distance</td>
</tr>
<tr>
<td>G-A distance</td>
</tr>
</tbody>
</table>

Figure -1 Length of acromian process measure by vernier calliper
In present study we found the length of the acromian was 45.0±0.52 mm’ the comparision of various parameters of acromian process with previous study done in table 5. The width (transverse diameter ) of the acromian projection in the current study shows much resemblance with previous studies. Edelson and Taitz\(^5\) had recorded that the thickness(breadth) and width(transverse diameter ) of Acromian projection have no relationship with pathological and physiological changes. We found thickness of acromian process was 7.8 mm which was compared with what recorded by Paraskevas et al\(^2\) (8.8 mm) that was higher as compared to the what observed by shilpi gosavi et al\(^8\) & Singh et al\(^7\) 6.9 mm and 6.6 mm respectively.

Sangiampong et al\(^{13}\) found pathology that reduce the subacromial space, causes impingement(compression) and the interval between the tendon of supraspinatous and the lower part of the acromian on front aspect , is decrease in position of 90 degree abduction with internal rotation .

Some of the parameters recorded of scapular morphometry shows difference with sex and age, and it clinically important.

**Conclusion**

The result of our study done on 74 dry scapula shows predominant type I scapula, we have measured various parameters of acromian process which was nearly similar to found in previous study ,the coracoacromian distance.
and coracoglenoid distance measured and compare with previous studies shows not much difference, this study helpful for orthopedics surgeon, physiotherapist, radiologist and other clinician for better understanding of anatomy and pathology of acromian process.

**Ethical Clearance** - Taken from Sumandeep Vidyapeeth committee

**Source of Funding** - Self

**Conflict of Interest** - nil

**References**


A Study Protocol for Checking Efficacy of Microsoft Kinect Azure for Evaluation of Spatial Parameters of Gait in Normal Healthy Population

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Abstract

Introduction: In healthy population, spatial parameters of gait such as stride length and step length are frequently linked with gait cycle but can be obstructive, time taking and difficult to measure. This study is to check the efficacy of Kinect azure for evaluating of spatial parameters of gait in normal healthy population. Kinect is accurate, unobstructive, low cost clinical gait analysis systems have many uses like diagnosis, monitoring, management and rehabilitation.

Method: The research has been designed as an observational study. The total of 132 participants will be taken from AVBRH, sawangi Meghe for study as per exclusion and inclusion criteria. With intervention the span of the study will be 6 months. It holds single period, concurrent validity evaluation comparing spatial gait parameters derived from the Kinect system.

Discussion: This study protocol aims to assess the efficacy of Microsoft Kinect azure for evaluation of spatial parameters of gait. The study’s expected outcome will concentrate on the evaluation of the usability of Kinect to assess spatial gait on healthy individuals.

Keywords: Kinect azure, projector, spatial gait analysis, healthy individuals.

Introduction

Research has shown the importance of measuring a person’s gait and that the parameters describing locomotion. A major rehabilitation goal is recovery of functional and independent community ambulation. A gait analysis can detect deviations, thus this may help in assessing rehabilitation potency. A gait analysis can also use to differentiate between disease and to determine general health and disease and accident threats such as detection of fall and prognosis between the elder individuals. Many individuals and the elderly suffer from gait impairments.

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Orchid ID:https://orcid.org/0000-0003-4484-8225
Designation –Vice Principle and Professor
This study also shows that stride-to-stride variation to evaluate velocity, speed and stride length are free of fall detection. Thus, it is convenient for recognizing high-risk individuals. Wearable sensors are proposed under recent studies. Such devices are small, portable, less expensive and lightweight (Figure 1). Despite their superiorities, wearable sensors have few drawbacks. Sensors should be placed securely and precisely, these sensors are obstructive in a way that it requires daily routine changes for subjects. It also requires maintenance of charging battery, uplinking data and sanitary treatment. Accurate, unobstructive, low-cost clinical gait analysis systems have many uses like diagnosis, monitoring, management and rehabilitation.

Recent evidence shows that the Kinect azure, which utilizes depth and image sensor data merge with AI algorithms to recognize anatomical landmarks without the need of sensors attached to the individual’s body (Figure 2). Furtherly, research reveals that the Kinect is able to validly evaluate stride dynamics in walking. Present devices are capable of precisely calculating spatial gait variables are much expensive, prologue and less handy.
The Microsoft Kinect azure is a cheap gaming device that has shown assurance as a clinical assessment tool. Thus, the objective of this study was to evaluate the concurrent validity and inter-day reliability of Kinect when assessing spatial parameters of gait.

**Aims And Objective**

**Aim:**
To assess stride length and step length in normal individual via Microsoft Kinect azure.

**Objective:**
1) To evaluate stride length in normal individuals by Microsoft Kinect azure.
2) To evaluate step length in normal individual by Microsoft Kinect azure.

**Methodology:**
This study will be conducted in the Department of Community Health Physiotherapy at Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, India, with the approval of Datta Meghe Institute of Medical Sciences, Institutional Ethics Committee, Deemed to be University.

**MATERIAL REQUIRED:**
1) Microsoft Kinect azure
2) Projector
3) Consent form

**Study setting:** Ravi Nair Physiotherapy College
**Study type:** observational type
**Sampling technique:** simple random
**Sample size:** 132 participants
**Study duration:** 6 months
**Study design:** This study will be carried out in the HumEn research lab of Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University.

Before inclusion, all the participants will be informed regarding the aim and procedure of research. Figure 3. Show’s the flow chart of the study.

**Figure :3**

**Outcome Measure:**
1. Spatial parameter of gait

**Participant selection:**

**Exclusion criteria:**
1) individual with abnormal gait.
2) Lower limb fracture.
3) Traumatic injuries of lower limb.
4) Neurological problem.

**Inclusion criteria:**
1) Normal individual without gait impairment.
2) Subjects willing to participate voluntarily.
3) Both male and female patients.

**Procedure:**
We’ll catch recording of 132 subjects (male as well as female) to participate. This study hold single period, concurrent validity evaluation comparing spatial gait parameters derived from the Kinect framework. Ask subject to walk down a line, at normal pace, in front of the Kinect sensor. Subject walk along a line , starting from the beginning point and slow down before reaching to the Kinect. The distances are compatible with the guidelines for achieving the highest data quality. This distance allows to record atleast 1 full gait cycle (i.e. complete stride) per walking trial for each limb that does not include the initial step and final step of the pathway.

Besides, age, gender, occupation, weight and height of the individual’s is recorded as a supplementary details for better data collection.

**Expected Result:**
The study’s expected outcome will concentrate on evaluation of spatial parameter of gait in healthy individual. After completion of study result will
calculated by systemic data analysis by randomized control trial.

**Discussion**

Study protocol aim is to evaluate gait parameter in normal individuals using Microsoft azure. Its expected result concentrate on evaluation of spatial gait parameter using kinect azure. This study help to find the efficiency of kinect azure used in evaluating gait parameter in healthy indivuals.

**ETHICS AND DISSEMINATION:**

The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review.

**Source of Funding:** There will be no direct support for this research from public and private organization. The department of physiotherapy, at Datta Meghe institute of Medical Science, Deemed to be university will provide material needed for research.

**Conflict of Interest:** Nil

**References**


Estimation of Interleukin-4 (IL-4) and Interleukin-6 (IL-6) Levels in Sera From Patients with Type 2 Diabetes Mellitus

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Abstract

Type-2 diabetes mellitus is the most common chronic metabolic disorder characterized by elevation of blood glucose concentration (hyperglycemia) due to defect in carbohydrate, lipid and protein metabolism. Cytokines have an important role in impairing insulin signaling and selective destruction of insulin-producing beta cells. Thus, the second type of diabetes can be characterized as an immune-mediated disease. This study aimed to evaluate the serum levels of Interleukin-4 (IL-4) and Interleukin-6 (IL-6) of patients with type 2 diabetes mellitus among cases from Wasit province-Iraq. A total of 90 randomly selected subjects from Wasit province – Iraq: (60) patients with T2DM, and (30) apparently healthy subjects with normal fasting blood sugar as a control group. Enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of interleukin-4 IL-4 and interleukin -6 in sera from patients with type-2 diabetes mellitus. The results of this study showed that IL-4 concentrations had a non-significant difference when compared patients with type-2 diabetes mellitus with the control group (154 ± 7.00 versus (vs) 151.49 ± 21, P-value (P) = > 0.05). While patients with T2DM revealed elevated serum levels of IL-6 compared to control group (B 637.1 ± 355.9 versus 266.3 ± 128.8, P = < 0.001).

Keywords: IL-6, interleukin-6; IL-4, interleukin-4; T2DM, type 2 diabetes mellitus, ELISA, the enzyme-linked immunosorbent assay.

Introduction

Recently, diabetes is classified as an epidemic disease due to its worldwide spread in varying proportions. Diabetes mellitus (DM) is defined as a heterogeneous metabolic disorder caused by hyperglycemia derived from either insulin action deficiency or impaired insulin secretion or both, which alters carbohydrate, protein, and fat metabolism. To date, the mortality rate of diabetes has increased to 1.5 million people making diabetes the 1st leading cause of death in the world. At a local setting, the prevalence of the disease in Iraq in 2012 was 10.9% depicting a serious rise in the number of T2DM patients.

One of the most common diseases in the world is type-2 diabetes mellitus. The increased acute immune response and pro-inflammatory cytokines were detected in diabetics in 1997. Since then, emerging evidence has shown that T2DM is a chronic inflammatory disease in which various stimuli, such as genetic or fatal metabolic pre-programming, over-nutrition or increased age, can increase levels of cytokines expressed. In which, the pattern of cytokine expression is changed, therefore, pro-inflammatory cytokines cause damage to pancreatic islet cells resulting in pro-inflammatory and protective cytokines imbalance.

Current studies are suggesting the possibility of inflammation being an important contributor to diabetes. This is due to the fact that inflammation can provoke changes in diabetes predominantly at the cellular
level, altering the functionality of tissues and cells demonstrating reactions of the inflammation including regulators, mediators, fibrinogen hs-CRP high sensitivity C reactive protein.

One family member of the cytokines is Interleukin 4 (IL-4) which is a typical cytokine of T helper type-2 (Th2) cells, could inhibit effect on the inflammation, decrease the production of pro-inflammatory cytokines and reduce the destructive enzymes through monocytes, and also plays a crucial role in the pathophysiology of T2DM. In addition to Interleukin-4 (IL-4), Interleukin-6 (IL-6) is a proinflammatory mediator cytokine biosynthesized by T-lymphocytes, macrophages, adipocytes and other sources such as endothelial cells, fibroblasts, and skeletal muscles.

On the other hand, IL-6 is responsible for many tasks such as controlling the activation and differentiation of T-lymphocyte responses and proinflammatory responses and also plays a role in the pathogenesis of autoimmune and inflammatory diseases, in the regulation of body weight, and in lipid metabolism.

This study aims to investigate the possible relationship between some cytokines (interleukin-4 and interleukin-6) and type 2 diabetes.

Material and Methods

Subjects

Patients group consist of 60 subjects with T2DM from Wasit province/Iraq (30 males and 30 females). Their ages ranged between 45–75 years. Control group comprised of 30 individuals (15 males and 15 females), apparently healthy subjects with normal fasting blood sugar and the controls were selected from Al- Karama Teaching Hospital (the local community of Wasit province – Iraq). Written consent was obtained by Al-Karama Teaching Hospital.

The criteria of the American Diabetes Association have been adopted in the diagnosis of T2DM in patients. This study excluded subjects with autoimmune diseases, cardiovascular diseases, acute and chronic inflammation.

Blood sampling

Venous blood (10 ml) has been collected in the infected tubes of patients and controls under sterile conditions between 08:30–10:30 am. Then the serum has been quickly frozen at (−20°C) and stored until further processed (Estimating the concentration of interleukins (IL-4 and IL-6) from patients with T2DM).

Estimation of IL-4 and IL-6 levels

Interleukin-4 and Interleukin-6 concentrations in sera were measured by ELISA using Human-IL-4-Mini ABTS-ELISA Development-Kit (Pepro-Tech, France) and Human IL-6 Mini ABTS-ELISA Development Kit (Pepro-Tech, France) as per the manufacturer’s instructions.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) or median (interquartile range). Differences between groups were tested with the Student’s t-test. The values of P < 0.05 were considered significant.

Results

The results of this determination as shown in Table 1, revealed that serum IL-4 concentrations displayed a non-significant difference in T2DM patients when compared with the control group (Diabetic patients 154.48 ± 7.00 compared with control 151.49 ± 6.21, P-value = 0.052). While, the serum concentrations of IL-6 revealed a significant difference in T2DM patients in comparison to controls (Diabetic patients 637.1 ± 355.9 compared with control 266.3 ± 128.8, P-value = 0.00013).
Table 1: Concentration of IL-4 and IL-6 in patients with T2DM and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-4 concentration pg/ml</th>
<th>IL-6 concentration pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>151.49±6.21</td>
<td>266.3±128.8</td>
</tr>
<tr>
<td>Diabetic Patients</td>
<td>154.48±7.00 ns</td>
<td>637.1±355.9 b</td>
</tr>
<tr>
<td>Probability P-value</td>
<td>0.052</td>
<td>0.00013</td>
</tr>
<tr>
<td>LSD c</td>
<td>6.3</td>
<td>11.43</td>
</tr>
<tr>
<td>Significant level</td>
<td>Non-significant</td>
<td>Significant</td>
</tr>
</tbody>
</table>

*a* Concentration of interleukin in pictogram / milliliter. Values are given as mean ± standard deviation of the mean (SD). 
*b* significant P = 0.00013 when compared diabetic patients group with control group. 
*c* LSD (11.43) Least significant difference is the value at a particular level of statistical probability (e.g. P ≤ 0.01 means with 99% accuracy) when exceeded by the difference between two varietal means for a particular characteristic. 
*ns* non-significant P-value = 0.052 when compared diabetic patients group with control group.

Regarding to IL-4 concentrations, males and females of diabetic patients displayed no significant differences when compared with controls (155.16 ± 8.15 versus 151.89 ± 6.75, P-value = 0.733; 153.79 ± 5.68 versus 151.10 ± 5.84, -value = 0.463) as in Table 2. Regarding IL-6 concentrations in T2DM males for this study showed significant difference comparing to controls (808.3 ± 296.0 versus 241.8 ± 9.0, P-value = 0.00022). No significant difference was showed in relation to female diabetic patients IL-6 levels in comparison to controls (466.0 ± 331.0 versus 290.9 ± 181.6, P-value = 0.065) as in Table 2.

Table 2: Concentration of serum IL-4 and IL-6 on the basis of gender (males and females) in diabetic and control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gender/ IL-4 concentration pg/ml</th>
<th>Gender/ IL-6 concentration pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control</td>
<td>151.89±6.75</td>
<td>151.10±5.84</td>
</tr>
<tr>
<td>Diabetic Patients</td>
<td>155.16±8.15 ns1</td>
<td>153.79±5.680 ns2</td>
</tr>
<tr>
<td>P-value</td>
<td>0.733</td>
<td>0.463</td>
</tr>
<tr>
<td>Significance</td>
<td>Non-significant</td>
<td>Non-significant</td>
</tr>
</tbody>
</table>
Concentration of interleukin in picogram/milliliter. Values are given as mean ± standard deviation of the mean (SD), nonsignificant P-value = 0.733 when compared diabetic male patients group with control male group, nonsignificant P-value = 0.463 when compared diabetic female patients group with control female group. nonsignificant P-value = 0.065 when compared diabetic female patients group with control female group. significant P-value = 0.00022 when compared diabetic male patients group with control male group.

Discussion

Type 2 diabetes is a metabolic disease caused by insulin resistance and characterized by abnormal metabolism of glucose, proteins, and lipids. In fact, Type 2 diabetes is described as an inflammatory disease, with cytokines playing an important role in its diseases. Inflammatory processes influence the development of insulin resistance and reduced insulin secretion by pancreatic beta cells. Cytokines act as signaling molecules for immune cells, especially in autoimmune diseases, so cytokines play their role in developing and activating these cells.

Table 1 revealed a non-significant difference of IL-4 levels when compared patients with T2DM to control. These results are in agreement with a study conducted by Nuhair and others (2018) on the residents of Nassryain population in Thi-Qar/ Iraq. Festa and others 2002, also mentioned the decreased production of IL-4 in human T-cells in diabetic patients. Moreover, the levels of IL-4 in the blood of diabetic patients demonstrated decreased values, this finding are disagreed with our results related to IL-4.

The differentiation activity of IL-4 plays an important role in the production of immunoglobulin, also IL-4 is described as a growth factor for β-cells. IL-4 developed by CD+4T-lymphocytes type Th2, after activation by antigen binding to the T-cell receptor, and also by activated mast cells and basophils. IL-4 down-regulates the production of IFN-γ by Th1 CD4 + T-lymphocytes on the β-cells, IL-4 has a growth factor role mediated through the development of soluble CD23. On monocytes, because of its pleiotropic activity, IL-4 induces an increased number of major histocompatibility complex (MHC) class II antigens.

The results of this study revealed elevated serum levels of IL-6 in patients with T2DM. These results are in agreement with Nuhair et al (2018), who mentioned that there was a significant increase in the levels of IL-6 among diabetic patients as compared with controls in a study conducted on the Nassryain population in Thi-Qar/ Iraq. Vidhate et al (2013) also mentioned that there was a significant increase in the levels of IL-6 among diabetic patients as compared with controls in a study conducted on Indian Population from Navi Mumbai. In addition, several studies have found an increase in serum IL-6 concentrations, however some studies reported no difference, or even decreased IL-6 levels.

IL-6 might play a significant role in IDDM etiopathogenesis. In general, high levels of IL-6 are usually observed in the blood of diabetics, which is known to play an important role in the development of certain vascular diseases and atherosclerosis in addition to its main role in increasing inflammation.

Chronic low-grade inflammation in obese people plays a significant role in the subsequent development of insulin resistance. This results in a triple increase in systemic cytokine levels including IL-6, and thus becomes a risk factor for T2DM. Some studies point to the important role that IL-6 plays in fat metabolism in general. In humans, the action of IL-6 is associated with increased plasma free-fatty acids (FFAs). Due to high concentrations of IL-6 and C-reactive-protein, which is a surrogate marker for IL-6 activity, these associations seem significant. In obesity, increased concentrations of IL-6 are observed. Further, an increase in the release of IL-6 by visceral adipose tissue was observed three to four times more than subcutaneous fat taken from obese and non-diabetic patients. Therefore the main sources of elevated plasma concentration IL-6 up to 2-3g/mL-1 in obese patients and T2DM are adipocytes and macrophages present in adipose tissue.

The primary cell kinds engaged in regulating peripheral insulin sensitivity and homeostasis of glucose, hepatocytes, skeletal muscle cells, and adipocytes, react differently to IL-6. Strong experimental proof supports IL-6’s capacity to decrease insulin sensitivity in hepatocytes by interfering with insulin signaling, while findings on adipocytes and skeletal muscle cells...
are not always coherent. Compared to other kinds of cells, the greater responsiveness of hepatocytes may be associated with the existence of membrane-bound IL-6R. It should be observed that these experiments are carried out primarily in vitro on cell lines and for a brief period, using supraphysiological of IL-6 levels much greater than those engaged in low-grade chronic inflammation.

In addition, the experimental circumstances may not strongly match acute pathophysiological circumstances in which IL-6 operates in synergy with other cytokines and during inflammatory responses mediates crosstalk between distinct kinds of cells and tissues. Moreover, the level of IL-6R and sgp130 is not assessed in targeted pathological models in these early studies, nor is IL-6 trans-signaling taken into consideration, which is particularly critical in the investigation of cells that lack membrane-bound IL-6R.

Further, Serum IL-6 levels have also been found to be associated with insulin resistance and diabetes. In nondiabetic older populations, and healthy, middle-aged, white populations. Schultz and others showed that higher IL-6 serum concentrations associated with enhanced opposition to insulin. Liu and others in 2007 showed that serum IL-6 levels were also found to be higher in people with impaired glucose tolerance, T2DM, or cardiovascular syndrome relative to those with ordinary glucose tolerance or those who did not fulfill metabolic syndrome standards. Recently, a big prospective study of postmenopausal females who participated in the WHI (Women’s Health Initiative) in the United States has revealed that high concentrations of IL-6 are also correlated with an increased risk of clinical diabetes. Thus, it is necessary to clarify the immune and physiological roles of this interleukin, and as far as we know there are very few studies that explain the reason for the high levels of interleukin-6 in patients with type 2 diabetes.

Regarding to IL-4 concentrations, males and females of diabetic patients displayed no significant differences when compared with controls (155.16 ± 8.15 versus 151.89 ± 6.75, P-value = 0.733; 153.79 ± 5.680 versus 151.10 ± 5.84, P-value = 0.463) as in Table 4.2. It is traditionally accepted that IL-4 has anti-inflammatory functions but may have multiple actions. IL-4 did not differ significantly between ChP and T2DM. Lower levels of IL-4 were observed in ChP + T2DM which is similar to another study, although IL-4 and TNF-α in their investigation correlated positively with each other in T2DM. Hence, a conclusive anti-inflammatory role for IL-4 is not forthcoming. To the best of our knowledge, the lack previous studies in people with T2DM on the basis of gender related to IL-4 and IL-6 levels or prediabetes does not permit direct comparisons with similar samples.

Concentrations of IL-6 in T2DM males showed significant difference comparing to controls (808.3 ± 296.0 versus 241.8 ± 9, (P-value = 0.00022). No significant difference was showed in relation to female diabetic patients IL-6 levels in comparison to controls (466.0 ± 331.0 versus 290.9 ± 181.6, P-value = 0.065) as in Table 2.

Serum IL-6 levels have also been established to be associated with insulin resistance and diabetes. In nondiabetic older populations and healthy, middle-aged, white populations. In females, it is possible that elevated IL-6 levels may largely reflect adipocyte activation. For instance, IL-6 and downstream CRP production may be associated with the corelease of other pathogenic substances arising from otherwise stimulated adipocytes. Other potential mediators of insulin resistance deriving from adipose stores include tumor necrosis factor-a, leptin, free fatty acids, and resistin. Nonetheless, under the assumption that elevated levels of IL-6 and CRP purely reflect altered adipocyte function, the ready availability of reliable and sensitive markers of this process may represent a novel approach for early identification of both obese and nonobese individuals at increased risk for the clinical development of this disease.

Conclusions

In this study, we have determined the concentrations of two interleukins (Interleukin-4 (IL-4) and Interleukin-6 (IL-6)) in sera from patients with type-2 diabetes mellitus using enzyme-linked immunosorbent assay (ELISA). From this study, we concluded that elevated levels of IL-6 may be related with type 2 diabetes mellitus whereas the levels of IL-4 may not be
This case-control study is investigating the association between type-2 diabetes mellitus and two important interleukins; Interleukin-4 and Interleukin-6. Determination of these interleukins in sera of type-2 diabetes mellitus patients is considered a vital assay to assess this association. Elevated levels of interleukins might play a significant role in type-2 diabetes mellitus etiopathogenesis. High levels of IL-6 may be associated with insulin resistance and diabetes. For a deeper view of the relationship between interleukins and type 2 diabetes mellitus, we propose large scale studies involving different regions of Iraq.

Conflicts of Interest: The authors declare complete freedom of any issue concerning conflict of interests related to this work.

Funding: This research has been carried out under Fundamental Research Grants Scheme (FRGS/1/2018/WAB05/UPSI/02/3) provided by the Ministry of Education of Malaysia. The authors would like to extend their gratitude to Universiti Pendidikan Sultan Idris (UPSI) that helped in grant management and provided infrastructures and facilities for this study. Special appreciation is also dedicated to University of Wasit for scientific consultation and financial support.

Acknowledgements: The authors would like to thank all patients and their family for help to perform this study.

Ethical Clearance: The authors declare that the approval of the completion of the research before the start of work by the Scientific Committee for the Biology Department in the Faculty of Sciences and Mathematics at the Universiti Pendidikan Sultan Idris (UPSI) and took the consent of all patients to conduct the research.

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1. Nuhiar, R. S., Salman, A. N., & Al-Rekaby, H. R. The some cytokines Levels (TGFβ1, IL-4, IL-6, and IL-17) in sera Patients with Diabetes Mellitus Type1, Type 2 in Nassiriya city. Journal of Thi-qar Science, 2018, 6(4), 42-48.
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Glial Fibrillary Acidic Protein (GFAP) and Cleaved Tau Protein (CTP) Biomarkers as a Forensic Tool for Detection and Assessment of Traumatic Brain Injury

Mohamed Kamel Mohamed, Mervat Hamdy Ab Elsalam, Eman Ab Elfattah Mohammed Elzohairy, Dina Sabry Ab Elfattah, Ehab Ab Elhalim Ab Elsalam

Abstract

Traumatic brain injury (TBI) is defined as a traumatically-induced structural brain injury or physiological disruption of brain function caused by an external force. Traumatic brain injury is a neuropsychiatric disorder that breaks down the remaining barriers between neurology and psychiatry. Several biomarkers have been developed to directly determine the pathology of the nerve cells in the central nervous system (CNS) when it is injured. This review recent research on brain injury biomarkers could be used for rapid and accurate diagnostics of TBI in easily accessible fluid.

Objectives: The purpose of this study was to assess utility of GFAP-BDP for the diagnosis of intracranial injury in patients with a positive clinical screen for head injury across the spectrum of TBI typically presenting to ED in Kasr- Alainy hospital.

Subjects & Methods: This prospective cohort study was based on the data collected from 90 cases presented to Kasr Al-Aini Hospitals Emergency Department, Cairo University, with history of traumatic brain injury through the period from April 2017 to Mars 2019. According to age, they were classified into 3 age groups; age group A (18-35 years), age group B (36-50 years) and age group C (> 50 years). Data were analyzed with respect to socio-demographic data, type of head injury, clinical presentations, radiological investigation, and management of TBI in relation to serum specific biomarkers level (GFAP, C-tau).

Results: The most common age group was age group B (18-35 years) (70%). Males were more common than females (71.1% and 28.9% respectively). The most common cause of trauma was fall from height (33.3%). Serum GFAP level and C-tau levels in studied groups show high significant correlation between them (p value <0.001).

Conclusion and Recommendations: The combination of the two biomarkers or more may be more useful than either biomarker in isolation for predicting intracranial lesions on CT scanning therefore decreases unwanted CT head scanning and radiological bad effect especially in young ages.

Keywords: Traumatic brain injury, Serum, GFAP, C-tau and CT scanning

Introduction

TBI is a common problem, called a silent epidemic because of a general unawareness of the condition. TBI is difficult to diagnose with imaging techniques, and there is no definite laboratory test to support the diagnosis. An undiagnosed case of TBI can result in premature
return to play with severe consequences or in a chronic neurodegenerative condition later in life.¹

Traumatic brain injury (TBI) is defined as a traumatically-induced structural brain injury or physiological disruption of brain function caused by an external force.²

An ideal laboratory test, detecting a brain injury–specific biomarker in one of the body fluids, would confirm or rule out the TBI, predict the outcome, and indicate when recovery is complete. This review recent research on brain injury biomarkers could be used for rapid and accurate diagnostics of TBI in easily accessible fluid.³

Head CT scan is the diagnostic modality of choice to evaluate patients for traumatic intracranial injuries. Although effective for detecting traumatic injuries that require observation or neurosurgical evacuation due to the widespread use of head CT scanning has been questioned due to potential adverse effects of radiation exposure, unnecessary emergency department resource use, and cost.⁴

Several biomarkers have been developed to directly determine the pathology of the nerve cells in the central nervous system (CNS) when it is injured.⁵ The blood–brain barrier (BBB), which normally is almost impermeable, can lose its integrity upon brain injury and allow the permeation of molecules into the blood.⁶

A number of patients who suffer from mild head injury later on develop significant disabilities. Biomarkers help identify and quantify the extent of injury and help predict the possible functional outcome of the patients.⁷

Materials and Method

Study population:

Data collected from 90 cases presented to Kasr Al-Ainy emergency department -according to specific criteria-during the period of the study. Egyptian origin and residence, more than 18 years, both sexes with history of traumatic brain injury were included in the study. While patients who had any pre-morbid non traumatic neurologic conditions or history of previous brain trauma were excluded from the study.

- Selected patients presenting within 24 hours of injury were subjected to draw (5cm) blood samples and dated to compare with time of injury. Serum samples were extracted and preserved at -20°C till chemical analysis.

- Selected patients were classified to groups according to age:
  1. Group (A) 18-35 years.
  2. Group (B) 36-50 years.
  3. Group (C) >50 years

- Another classification according to Glasco Coma Scale into:
  2. Moderate TBI (GCS 9-12). The study cannot depend on CT head as it is not informative.

- All patients had at least one CT head scan at admission time.

- Time of traumatic brain injury time obtained from patients themselves, patient relatives, ambulance crew, and others (referral letter, police report).

- The study will measure the level of GFAP and Cleaved Tau Protein (CTP) by ELISA a proprietary mouse monoclonal antibody for solid phase immobilization, and a proprietary polyclonal rabbit antibody for detection.

Data Collection

The current study sample collection process is designed to be taken in first 24 hours after trauma causing brain trauma. Negative CT head patients presented to emergency department with history of TBI must be observed at least 24 hours and another CT head may be further more indicated. Study of brain specific biomarkers will help doctors to decrease unnecessary CT head, decrease period of hospital stay and give them protection form medical liability if deterioration or death after uncalculated discharge has been occurred.

It also, divides 24 hours after trauma (total time of sample collection) to three subgroups to give more prediction accuracy to diagnose and evaluate TBI using brain specific biochemical markers level in relation to
delay time from trauma to sample collection.

The data will be formulated according to the data revealed from:

§ Patients primary survey (clinical examination and radiological CT scan)

§ Laboratory investigations (serum GFAP and C-tau levels). All cases presented with history of TBI within 24h of the injury.

I. Demographic data:
• Age: classified according to group A, B, and C.
• Sex: Males & Females.

II. Clinical picture:
• GCS score: 13-15 points, 9-12 points.
• Pupil light reflex: Normal, Sluggish.
• Vomiting: Yes, No.
• Severity of injury: Mild, Moderate.

III. Radiological investigations:
• CT head scan findings: Positive, Negative.
• MRI brain: Not indicated, Indicated.

IV. Lab investigations:
• C-tau level: <100, 100-149, 150-199, 200-300, >300.

V. Management: Conservative, surgical.

Ethical considerations

The study approval was taken by the ethical review committee of medical research, Faculty of Medicine, Cairo University, Egypt and informed consent forms were obtained from all participants. The study information including the purpose and details were explained to participants of both groups.

Results

Table 1 showed that 11.1% (10 patients) of cases had GFAP level below 200 and 45.6% (41 patients) of cases had level between 200-499, 27.8% (25 patients) of cases had level between 500-999, 14.4% (13 patients) of cases had level between 1000-2000 and finally 1.1% of cases had level more than 2000. The results showed that 5.6% (5 patients) of cases had C-tau level below 100 and 45.6% (41 patients) of cases had level between 100-149 then 18.9% (17 patients) of cases had level between 150-199 then 20.0% (18 patients) of cases had level between 200-300 finally 10.0% (9 patients) of cases had level more than 300

<table>
<thead>
<tr>
<th>GFAP level (pg./ml)</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>10</td>
<td>11.1</td>
</tr>
<tr>
<td>200-499</td>
<td>41</td>
<td>45.6</td>
</tr>
<tr>
<td>500-999</td>
<td>25</td>
<td>27.8</td>
</tr>
<tr>
<td>1000-1999</td>
<td>13</td>
<td>14.4</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>603.9±443.9</td>
</tr>
<tr>
<td>Median (Range)</td>
<td></td>
<td>477.0(93-2995)</td>
</tr>
</tbody>
</table>
Continued... Table 1: Serum GFAP and C-tau levels among studied cases

<table>
<thead>
<tr>
<th>C-tau LEVEL (pg./ml)</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>5</td>
<td>5.6</td>
</tr>
<tr>
<td>100-149</td>
<td>41</td>
<td>45.6</td>
</tr>
<tr>
<td>150-199</td>
<td>17</td>
<td>18.9</td>
</tr>
<tr>
<td>200-300</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>&gt;300</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>184.7±92.6</td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>174.0(78.0-597.0)</td>
<td></td>
</tr>
</tbody>
</table>

Comparing pupil light reflex, GCS and management hospital stay, with GFAP level among studied cases, there was statistical difference between mean GFAP biomarker level which was highly significant (p value 0.001 0.001, 0.005, 0.023).

Table 2: GFAP level in relation to pupil, vomiting, GCS and management (mean ± SD) using ONE way ANOVA

<table>
<thead>
<tr>
<th>GFAP</th>
<th>Mean ±SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>550</td>
<td>447</td>
<td>424</td>
<td>93</td>
<td>2995</td>
</tr>
<tr>
<td>Sluggish</td>
<td>769</td>
<td>401</td>
<td>626</td>
<td>200</td>
<td>1732</td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>541</td>
<td>324</td>
<td>477</td>
<td>93</td>
<td>1425</td>
</tr>
<tr>
<td>Yes</td>
<td>707</td>
<td>582</td>
<td>477</td>
<td>132</td>
<td>2995</td>
</tr>
<tr>
<td>GCS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-15</td>
<td>547</td>
<td>439</td>
<td>427</td>
<td>93</td>
<td>2995</td>
</tr>
<tr>
<td>9-12</td>
<td>815</td>
<td>405</td>
<td>639</td>
<td>348</td>
<td>1732</td>
</tr>
<tr>
<td>Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative</td>
<td>574</td>
<td>494</td>
<td>418</td>
<td>93</td>
<td>2995</td>
</tr>
<tr>
<td>Surgical</td>
<td>664</td>
<td>322</td>
<td>590</td>
<td>219</td>
<td>1732</td>
</tr>
</tbody>
</table>

*P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant
Table 3 Comparing management with C-tau level among studied cases, there was statistical difference between mean C-tau which was highly significant (p value <0.001)

<table>
<thead>
<tr>
<th>C-tau</th>
<th>Mean ±SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>182.7 95.6</td>
<td>142.0 78.0</td>
<td>597.0 0.464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sluggish</td>
<td>190.9 84.4</td>
<td>154.5 105.0</td>
<td>374.0 0.464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>176.6 80.8</td>
<td>144.5 78.0</td>
<td>459.0 0.398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>198.1 109.3</td>
<td>154.5 84.0</td>
<td>597.0 0.398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-15</td>
<td>179.6 94.1</td>
<td>141.0 78.0</td>
<td>597.0 0.134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-12</td>
<td>203.7 86.4</td>
<td>163.0 115.0</td>
<td>374.0 0.134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative</td>
<td>180.0 96.0</td>
<td>144.5 78.0</td>
<td>597.0 &lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td>194.0 85.8</td>
<td>153.0 108.0</td>
<td>374.0 &lt;0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: C-tau level in relation to pupil, vomiting, GCS and management (mean ± SD) using ONE way ANOVA

*P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant

Table 4 Comparing CT head and severity of injury with GFAP level among studied cases, there was statistical difference between mean GFAP level which was significant p value (<0.001, 0.001).

Table 4: GFAP level in relation to CT head, severity of injury, MRI brain and head fracture (mean ± SD) using ONE way ANOVA

<table>
<thead>
<tr>
<th>GFAP</th>
<th>Mean ±SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C T head</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>811 483</td>
<td>612 219</td>
<td>2995 0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>333 144</td>
<td>342 93</td>
<td>733 0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>547 439</td>
<td>427 93</td>
<td>2995 0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>815 405</td>
<td>639 348</td>
<td>1732 0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not indicated</td>
<td>611 455</td>
<td>475 93</td>
<td>2995 0.795</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicated</td>
<td>548 359</td>
<td>483 200</td>
<td>1497 0.795</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant
Table 5 Comparing CT head with C-tau level among studied cases, there was statistical difference between mean C-tau level which was significant (p value 0.026).

Table 5: C-tau level in relation to CT head, severity of injury, MRI brain and head fracture (mean ± SD) using ONE way ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Mean ±SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C T head</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>205.1 106.3</td>
<td>163.0 102.0</td>
<td>597.0</td>
<td></td>
<td>0.026*</td>
</tr>
<tr>
<td>Negative</td>
<td>158.0 62.8</td>
<td>139.0 78.0</td>
<td>358.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>179.6 94.1</td>
<td>141.0 78.0</td>
<td>597.0</td>
<td></td>
<td>0.134</td>
</tr>
<tr>
<td>Moderate</td>
<td>203.7 86.4</td>
<td>163.0 115.0</td>
<td>374.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not indicated</td>
<td>182.3 94.2</td>
<td>144.5 78.0</td>
<td>597.0</td>
<td></td>
<td>0.261</td>
</tr>
<tr>
<td>Indicated</td>
<td>204.4 80.3</td>
<td>197.5 115.0</td>
<td>358.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant

Table 6 showed Pearson correlation between GFAP level and C-tau level in studied groups there was high significant correlation between them (p value <0.001).

Table 6: Pearson correlation between GFAP and C-tau TBI biomarkers level in studied cases.

<table>
<thead>
<tr>
<th></th>
<th>Pearson Correlation</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAB level and C.tau level (pg./ml)</td>
<td>0.402</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

**Discussion**

Regarding GFAP level in studied cases, the study showed that 11.1% (10 patients) of cases had GFAP level below 200 and 45.6 % (41 patients) of cases had level between 200-499 then 27.8 % (25 patients) of cases had level between 500-999 then 14.4 % (13 patients) of cases had level between 1000-1999 finally 1.1 % (1 patients) of cases had level more than 2000. Most of studied cases had a level 200-499.

This is in agreement with 8 who reported that GFAP levels were significantly higher in those with evidence of traumatic pathoanatomic CT features when compared subjects with a negative head CT scan. (CT negative, mean GFAP 0.26±0.41 ng/mL; CT positive, mean GFAP 2.88±3.74 ng/mL; p<0.01)
Regarding C-tau level in studied cases, the study showed that 5.6% (5 patients) of cases had C-tau level below 100 and 45.6% (41 patients) of cases had level between 100-149 then 18.9% (17 patients) of cases had level between 150-199 then 20.0% (18 patients) of cases had level between 200-300 finally 10.0% (9 patients) of cases had level more than 3000. Most of studied cases had a level 100-149.

9 reported that the combination of the two biomarkers or more may be more useful than either biomarker in isolation for predicting intracranial lesions on CT scanning.

Regarding pupil light reflex, GCS, management, duration of hospital stay with GFAP level among studied cases, there was statistical difference between mean GFAP biomarker level which was highly significant (p value 0.001 0.001, 0.005, 0.023).

The current study is in agreement with 10 who studied multivariable prognostic analysis in traumatic brain injury as expected, both the GCS motor score and pupil response are powerful independent predictors of outcome. GCS also can predict the line of treatment and estimated period of hospital stay.

11 who studied Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients the study showed similar results that by pupil light reflex examination of TBI presented cases, reflex was normal in 80% of patients. The prediction value of pupil light reflex test was higher than other clinical variables such as age, occupation, and vomiting in diagnosis and evaluation of traumatic brain injuries.

The study concluded that, patients with lower GCS had significantly higher level of serum C-tau protein and were associated with poor outcome. Correlating the serum C-tau levels and the GCS points at which it is significant may guide us further in qualitatively analysing the significant serum tau levels and throw some light on its role in projecting TBI prognosis.

9 who studied acute biomarkers of traumatic brain injury, the mean C-tau biomarker levels differ between moderate to severe TBI (GCS 3–12) and mild TBI (GCS 13–15) and also differ between complicated mild TBI (GCS 13–15 with abnormal cranial CT) and uncomplicated mild TBI.

Regarding CT head and severity of injury with GFAP level among studied cases, there was statistical difference between mean GFAP level which was significant p value (<0.001, 0.001) Table (51).

Our results are in accordance with 8 who proved that, the ability of the GFAP level to discriminate between patients with mild and moderate-to-severe injuries, as measured by the AUC, was 0.87 (95% CI, 0.81–0.93). The discriminatory ability of GFAP in assessing mild-moderate versus severe injury was 0.84 (95% CI, 0.77–0.91).

This is results are in accordance to 12 who suggested that, in patients with traumatic intracranial lesions on CT head scan, GFAP levels were significantly elevated (median, 0.588ng/mL), compared with those without lesions (median 0.033ng/mL).

In the present study, mean serum C-tau level in CT positive group was significantly higher compared to CT negative group. These results signify that this increase in serum C-tau level may be used to discriminate between patients with intracranial lesions and those without intracranial lesions, irrespective of the severity of injury.

13 mentioned that the increased serum C-tau level in severe head injured patients was associated with compromised blood–brain barrier (severe TBI). In contrast to 14 the study reported that CSF C-tau levels and found its levels were elevated 1,000-fold in TBI patients (1,519.6 ± 3,019 ng/ml) as compared to controls (0.031 ± 0.11ng/ml). Thus, C-tau may prove to be a promising molecule in severe TBI, provided more number of studies with highly sensitive detection methods in serum and CSF are undertaken.

Regarding to Pearson correlation between GFAP level and C-tau level studied groups there was high significant (p value <0.001) and low positive correlation (R=0.402).The second novel finding of this study is that the consideration of both biomarkers together improves the sensitivity and specificity for TBI diagnosis compared with each considered alone. Both serum GFAP and C-tau levels are powerful independent predictors of TBI outcome.
The current study showed that there is a positive correlation between high serum GFAP and C-tau protein levels and early diagnosis of TBI taking all other variables in consideration. In order to determine whether there was also a positive correlation between serum C-tau protein levels and patient outcome, the present study compared the serum C-tau protein levels in patient presented with history of TBI using clinical and radiological variables to add more prediction accuracy to studied brain specific biochemical markers. The study focused on the serum GFAP and C-tau protein levels during first 24 hours after TBI, showed the most significant differences among the three groups and thus was the most representative.

Conclusion and Recommendations

Serum GFAP level had greater value than serum C-tau in detection of intracranial lesion. There was a statistically significant but weak correlation between serum levels of C-tau and GFAP. Serum GFAP and C-tau levels in CT positive group were significantly higher compared to CT negative group. GCS had a strong significant relation with CT head findings and GFAP level. TBI biomarkers (GFAP and C-tau) have shortage in expression kinetics making them difficult (moving targets) to develop as reliable diagnostics. Blood samples used for TBI biomarkers measurement should be taken at multiple consecutive times after head trauma.

Declarations:

- **Funding**: None.
- **Acknowledgements**: None.
- **Conflict of Interest**: The authors declare that they have no competing interests.
- **Availability of data and materials**: Data will not be shared with public access.
- **Consent for publication**: Consent forms were given and signed by all subjects prior to participation

References


Cognitive Impairment and Its Impact on Quality of Life in Rural Indian Female after Stroke: A Cross Sectional Study Protocol

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Abstract

Introduction: Stroke represented growing social, health care and economic problems. In 2005 vascular cognitive impairment a condition that can be found in 20-30% of stroke patient¹. Now in 2018-19 over 50 percent of stroke survivors have reported cognitive impairment 6 months after stroke and are associated with poorer quality of life which increase disability. Cognitive functions is identified as a top priority for stroke research. Cognitive impairment affects inadequate ability to focus on the job, recall, understand, prepare, use knowledge, initiate and stop the operation and solve problem. a stroke impaired cognitive function including focus, memory, vocabulary, executive function, perception and orientation of space. Because of abnormality in functional independence and other abnormalities in higher function, cognitive impairment may lead to affect independence. it increases the death ratio, abnormality. Stroke impacts wellbeing dramatically on health system resulting in high costs, and is also considered a global public health problem due to severe disabilities, functional deficiencies and reduced quality of life.

Method: The corrective study is assessing the cognitive impairment and the quality of life. The cognitive impairment will be measured with the use of the MoCA and QoL will be measured by stroke specific quality of life questionnaire. Female stroke patient with age in between 45-65 year who was diagnosed by the physician is included in the study.

Discussion: Stroke is a prevalent condition which affects most of the Indian population. Most studies are done on stroke including male and females both. Many studies have concentrated on cognitive disability after stroke and quality of life in males but no research is available in rural Indian females. The need for the research is therefore to establish the prevalence of cognitive disability in females and their effect on quality of life after stroke.

Key Words: Rural Indian females, Stroke, Cognitive impairment, Quality of life, MoCA, and SS-QoL scale.

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Introduction

Stroke is a leading cause of death and disability in India¹. It represented growing social, health care and economic problems. In 2005 vascular cognitive impairment a condition that can be found in 20-30% of stroke patient². Now in 2018-19 over 50 percent of stroke survivors have reported cognitive impairment 6 months after stroke and are associated with poorer
quality of life which increase disability

The cognitive impairment after stroke is disturbed in any cognitive domain after stroke, executive function, memory, language, visuo-spatial function, visual-construction ability or global cognitive function. Hence, cognitive function is identified as a top priority for stroke research. Cognitive impairment affects inadequate ability to focus on the job, recall, understand, prepare, use knowledge, initiate and stop the operation and solve problem. A stroke impaired cognitive function including focus, memory, vocabulary, executive function, perception and orientation of space. The word cognitive impairment post-stroke is used to describe both mild cognitive impairment and dementia that either occurs 3-6 months after stroke incident. In addition, An approximate 16.9 million stroke cases occurred globally in 2010.

We do not know how many patients with strokes display deterioration or worsening of their cognitive impairment over a period of several years, and we do not have clear prognostic clues to classify those more likely to deteriorate. The estimates modified stroke prevalence range 84-262/100000 in rural areas, and 334-424/100000 in urban areas.

The definition of post-stroke cognitive impairment usually refers to disorders that arise after neuro-radiological examination after symptomatic stroke with associated ischemic finding. The post-stroke depression was found to be cognitive impairment related. It is believed to be linked mortality, reducing functional outcome and quality of life, and may be correlated with multiple factors and psychological mechanisms. Cognitive deficits are a negative prognostic factor that affects behavior and personality. The method such as Montreal Cognitive Assessment is commonly used clinically for examining cognitive disorder. Because of abnormality in functional independence and other abnormalities in higher function, cognitive impairment may lead to affect independence. It increases the death ratio, abnormality.

The physical, social and psychological effects of this disease are severe – approximately 90% of survivors have some kind of impairment. Quality of life in relation to health refers to all types of quality of life affected by diseases. Stroke impacts wellbeing dramatically on health system resulting in high costs, and is also considered a global public health problem due to severe disabilities, functional deficiencies and reduced quality of life.

**Need of The Study/ Rationale**

Stroke is a prevalent condition which affects most of the Indian population. Most studies are done on stroke including male and females both. Many studies have concentrated on cognitive disability after stroke and quality of life in males but no research is available in rural Indian females. The need for the research is therefore to establish the prevalence of cognitive disability in females and their effect on quality of life after stroke.

**Aim and Objective**

**Aim:** To assess the effect of Cognitive Impairment on quality of life in Rural Indian women after stroke.

**Objective:**

1. To find out the Cognitive Impairment in females after Stroke.
2. To find out the impact of cognitive impairment on the Quality Of Life of rural females after stroke.

**Material and Methodology**

Ethical approval will be obtained from the Institutional ethical committee. 40 participants will be selected randomly specially females and assessed for cognitive impairment and quality of life after obtaining consent form.

**Material:**

1. Couch
2. Chair with hand support
3. Immobilizer belt
4. Table
5. Pen

**Method:**

The research project will be conducted in Ravi Nair College of physiotherapy with rural population. It is cross-sectional observational study. All female patients...
who were diagnosed as having stroke and who fulfilled
the inclusion and exclusion criteria included in the study.

The corrective study is assessing the cognitive
impairment and the quality of life. The cognitive
impairment will be measured with the use of the MoCA
and QoL will be measured by stroke specific quality of
life questionnaire.

Mixed etiologies subject is excluded from the study.

**Instrumentation:**

MoCA , and SS-QoL scale is use as instrument.
The study selected because:

2. It is simple and convenient
3. Does not involve expensive technology or not to costly
4. It is time efficient to perform
5. Easy to understand
6. Reliability of MoCA is 0.75-0.96 , and SS-QoL is 0.65-0.99

**STUDY DESIGN:** Observational study.

**STUDY SETTING:** Ravi Nair College of Physiotherapy, Sawangi(M), Wardha

**PARTICIPANTS:**

**Inclusion and Exclusion Criteria:**

Inclusion criteria:

1. Female stroke patient
2. Age in between 45-65 year who was diagnosed by the physician is included in the study.

Exclusion criteria:

5) The patient having mix – etiologies
6) Elderly female above 67 year
7) Any other psychological disorder is excluded from the study.

**VARIABLES:**

1. MoCA Scale
2. SS-QoL Questionnaire.

**Data Sources/ Measurement:**

Ethical clearance will be obtained from institutional ethical committee (IEC). Participant will be selected by simple random technique. Inclusion and exclusion criteria will be implemented assessment during the hospital stay or within 3 month of stroke. To assess the different aspect of cognitive functions each participant tested approximately 1 and ½ hours session.

MoCA is commonly used method for cognitive evaluation. Eventually the MoCA returns a judgment-based score. The first only requires verbal input and involves orientation, memory and attention assessment. The second portion assesses the naming skills to obey verbal and written orders, writes a sentence randomly and copies a complex polygon.Since the MoCA includes divisions there is no limit of sub-domains. Total total score is 30 and total administration time is around 10-15 min.A cutoff <24 is used to define cognitive impairment.

With the SS-QoL we assessed Life Value

We applied the stroke Specific quality of life scale this method to measure the Quality of Life. The SS-QoL is a particular instrument used to measure health-related quality of life among individuals suffering stroke.It holds 49 objects in 12 domains. Higher values suggest a better quality of life linked to health.

**Data Analysis:**

Data analyzes are carried out using concise and infererensive statistics, using unpaired chesquare research students. The program used in the study will be the SPSS 24.0 version, the praphade prism 7.0 version and the degree of significance < 0.005 (p>0.005 m) is considered.

**STUDY SIZE:** 40

**STATISTICAL METHOD:** Simple Random Sampling

**Result**

Upon completion of the study results, statistical analysis will be estimated.
The result could show the impact on cognitive impairment and quality of life of post stroke patients in rural Indian females. or

The result could not show the impact on cognitive impairment and quality of life of post stroke patients in rural Indian females.

**Discussion**

Stroke is a prevalent condition which affects most of the Indian population. Most studies are done on stroke including male and females both. Many studies have concentrated on cognitive disability after stroke and quality of life in males but no research is available in rural Indian females. The need for the research is therefore to establish the prevalence of cognitive disability in females and their effect on quality of life after stroke.

**Limitations:**

It might be difficult to get convince patient for being a part of this study.

**Implication:**

Outcome of the study shall help the geriatric population to get aware about their condition and health the researcher to plan the treatment protocol.

**Generalizability:**

study not yet done.

**Ethics and Dissemination:**

The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review

**Source of Funding:** There will be no direct support for this research from public and private organization. The department of physiotherapy, at Datta Meghe institute of Medical Science, Deemed to be university will provide material needed for research.

**Conflict of Interest:** Nil

**References**


Source of Funding: There will be no direct support for this research from public and private organization. The department of physiotherapy, at Datta Meghe institute of Medical Science, Deemed to be university will provide material needed for research.

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Conflict of Interest: Nil

References
The Accuracy of Intrinsic Compression Ultrasound Elastography (E-Thyroid) in Differentiating Benign From Malignant Thyroid Nodule

Najlaa Hanoun

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Abstract

Background: Ultrasound elastography (USE) is an ultrasound-based technique that addresses the biomedical properties of the nodules. Based on the stiffness of the tissue this technique differentiates between the benign nodules from the malignant ones where the hard and soft tissue is determined by the high or the low elasticity values respectively. The present study aimed to evaluate the efficacy of the E-thyroid in the differentiation of malignant and benign thyroid nodules and also a cut off ECI value using fine-needle aspiration as the diagnostic standard of reference

Methods: The current prospective study was done on 80 patients in the radiology department in Al Yarmouk teaching hospital in Baghdad city from April 2018 to April 2019. All the patients referred from the outpatient clinic. All the patients were examined sonographically starting with B mode ultrasound then elastoscan examination was done.

Result: The study result showed that among the 80 patients 65 had a benign nodule and only 15 were diagnosed with a malignant thyroid nodule. The ECI value and the histopathological result showed a significant agreement in the result and a cut off ECI value of 4 were shown to be effective to diagnose the presence of malignant thyroid nodule.

Conclusion: Overall, the present study showed that USE can be effectively used to differentiate between the malignant and benign thyroid nodules and being a safe method this can be used widely. However, the result of this study needs further validation with a larger study population.

Keywords: Ultrasound Elastography, (E-Thyroid), Benign Thyroid Nodule, Malignant Thyroid Nodule, Elasticity, ECI value, Cut-off

Introduction

Thyroid nodules are common findings and can be found in almost 50% of the unselected population on ultrasound imaging. Although in most of the cases these nodules are benign, however, approximately 4% to 6.5% of all the thyroid nodules are malignant (1).

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Ultrasonography being a noninvasive technique provides significant information regarding the pathological features of the nodules. Studies have shown that certain features as observed in the US findings such as microcalcification, solid composition of the nodules,
wider shape and irregular margins certainly indicate the presence of malignancy (3). In contrast, smooth margins with round shape, presence of peripheral vascularity, and cystic composition are the main features of benign nodules (4).

However, it was also reported that only US finding cannot be regarded as the sole technique for differentiation between malignant and benign nodules. To overcome the disadvantages of the US findings FNA technique was used to rule out the presence of cancer in thyroid nodules. Although FNA is a safe procedure the pain and the discomfort faced by the patient made this technique self-limiting (5).

In this context, the need for a technique was warranted that can overcome the disadvantages of ultrasonography and on the other hand, can effectively differentiate between the malignant from the benign nodules. Ultrasound elastography (USE) has recently been introduced in the examination of thyroid nodules. It is an ultrasound-based technique that addresses the biomedical properties of the nodules. Based on the stiffness of the tissue this technique differentiates between the benign nodules from the malignant ones where the hard and soft tissue is determined by the high or the low elasticity values respectively (6).

This method takes advantage of the changing elasticity of the soft tissues resulting from physiological or pathological conditions. E-thyroid uses the pulsations generated from the adjacent common carotid artery and thus it eliminates the need for manual compression of the transducer. This technique offers greater consistency in the ElastoScan image. However, different studies have reported the different diagnostic capacity of this technique. This difference in the result was mainly due to inter as well as intraobserver agreements, biased selection of images and subjective scoring. An elasticity contrast index (ECI value) is provided by this technique which is calculated by comparing the elasticity value of the nodule with normal tissue present within the ROI.

Previously this technique was used in the differentiation of malignant and benign breast masses and liver fibrosis staging (7),(8). Very recently this technique was started using in the differentiation of benign and malignant thyroid nodules. However, very few studies were conducted in this filed. In the present study, we aim to evaluate the value of E-thyroid in the differentiation of malignant and benign thyroid nodules & determining the cut-off value of ECI by using fine-needle aspiration as the diagnostic standard of reference.

Method and Materials

Selection of study population

This prospective diagnostic study was conducted in the radiology department of Al Yarmouk teaching hospital in Baghdad city from April 2018 to April 2019. Before the beginning of the experiment, the ethical clearance for the study was taken from the ethics committee of the al-mustansirhya college of medicine

All the patients who have visited the outpatient departments with the complication of single or multiple thyroid nodules were included in this study. A total of 80 patients who visited this hospital in the study period were included in the study. All the patients were examined sonographically starting with B-mode ultrasound then elastographic examination was done. Before enrollment, informed consent was obtained from each participant.

The exclusion criteria for the study were as follows:

(1) TR-1 nodule (0 points), benign

(2) Cystic component >20% of the nodule volume.

(3) Large nodules occupying >75% of thyroid lobe volume because insufficient surrounding normal thyroid tissue to be used as reference

(4) Nodules with the calcified shell.

Procedure:

Ultrasound & Elastoscan examination was performed using RS80A of Samsung equipped with E-thyroid software, using a 5 to 12 MHz linear transducer. At first, patients were instructed to lay in the supine position and then they were first examined by B-mode in at least 2 plain (axial & sagittal) to illustrate the different characteristics of thyroid nodule(s) such as maximum nodule dimensions, echogenicity, alignment, and calcification the nodules. Then the nodules were scored according to above TI-RAD system from (TR2 to TR5) while asking patient to hold breath for awhile a last scan data acquired from the nodule after delineation.
of its boundaries by operator, a multiple ECI value (Elasticity contrast index) were obtained and computed interactively and displayed on monitor, the largest value is assigned for that nodule for analysis of diagnostic accuracy.

After that, the position of each selected nodule(s) concerning the CCA is divided to either close or intrinsic to assess the possibility of the change of ECI value in relation to nodule position from the source of compression. A higher ECI value suggested a stiffer nodule with increased possibility of malignant tissue.

FNA examination under U/S guidance was used as the diagnostic standard of reference.

Statistical analysis:

The statistical analysis was then conducted on the results obtained. The data obtained by the histological analysis and the US data were compared and a p-value < 0.05 was regarded as statistically significant.

Result and Interpretation

The present study included a total of 80 patients among which 64 patients (80%) were female and 16 (20%) were male indicating a female predominance. Among these patients, only 7.5% were below 29 years of age group. The maximum of the female patients was in the 40 years to 49 years age group (31.3%). On the other hand maximum, male patients were in the 50 years to 59 years of age (10%). Overall, the highest number of patients was in the 40-49 years age group (37.5%) and the maximum of the patients were female. The statistical analysis showed a significant association between the age and gender of the patient with the prevalence of the thyroid nodule formation (Table 1).

No statistically significant association was reported for tumor position with the benign and malignant nature of the nodules (Table 2). (Table 3) depicts the correlation of histopathological results with the ECI value. This result indicates a highly significant association between the ECI value with the Histological findings. In the study, 81.4% of the nodules were found to be benign by histopathological examination compared with the ECI value which showed 90% as the benign nodules. On the other hand, 18.8% nodules were found to be malignant by histopathological finding and 10% of the nodules were found to be malignant by ECI value. The ECI value of 4 was set as the cut off for malignant nodule identification.

<table>
<thead>
<tr>
<th>Table (1): Distribution of patients according to gender and age groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td><strong>Male</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>%</td>
</tr>
</tbody>
</table>

MCP ≤ 0.05 S
Table (2): the position of nodule according to the type of tumor

<table>
<thead>
<tr>
<th>Histopathological result</th>
<th>position of the nodule in correlation to CA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>close</td>
<td>intrinsic</td>
</tr>
<tr>
<td>Benign</td>
<td>9</td>
<td>56</td>
</tr>
<tr>
<td>Malignant</td>
<td>11.3</td>
<td>70.1</td>
</tr>
<tr>
<td>Total</td>
<td>7.5</td>
<td>11.2</td>
</tr>
</tbody>
</table>

MCP≥ 0.05 NS

Table (3): correlated between Histopathological Result and ECI value

<table>
<thead>
<tr>
<th>Histopathological Result</th>
<th>the ECI value</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.9-1.9</td>
<td>2.0-2.9</td>
</tr>
<tr>
<td>Benign</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>%</td>
<td>32.5%</td>
<td>41.4%</td>
</tr>
<tr>
<td>Malignant</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>35</td>
</tr>
</tbody>
</table>

MCP≤ 0.01 HS
Cut-off: 4.0

Discussion

Ultrasound elastography (USE) is an emerging non-invasive diagnostic tool that measures the differences of stiffness between malignant and benign lesions. The malignant tissue is stiffer compared with the normal tissue making the nodules less elastic compared to the benign nodules. Presently clinical knowledge on the calcified nodules suggests not to use the USE technique on calcified nodules because of the higher number of false-positive results. Previous studies have pointed out that this calcification results in increased stiffness even in benign nodules also resulting in false-positive results.
In the present study, a total of 80 patients were included and Ultrasound elastography was performed on these patients. A female predominance was observed in the study. The result indicated that the presence of a thyroid nodule has a significant correlation with the age and the gender of the patient.

Previous studies have pointed out that carotid artery pulsation can be used as the compression source and can significantly reduce the inter and intraobserver variability. This removes the hindrance of the carotid artery pulsations. Based on the objective scoring obtained by using a unique algorithm and an elasticity contrast found within the nodule, users can obtain reliable data on the degree of the elasticity (9),(10).

Based on these findings in the present study also the distance from the carotid artery was investigated. Most of the benign nodules were intrinsic in position to the carotid artery while in the malignant group total of 9 nodules were intrinsic to the carotid artery. However, no statistical significance was reported for this result. In another study conducted by Lim and Kim, it was reported that the finding by the USE is significantly associated with the distance from the carotid artery. The nodule that is closer to the carotid artery has an ECI value lower than the nodules present far from the artery. They have also instructed to remeasure the extreme lower ECI value before considering it as a benign nodule if the distance of the nodule is closer to artery 2.

In another study by Kim et al., a group of 165 patients was examined using the Accuvix XG machine supported with an E-Thyroid™ software. In this study, 154 nodules were found to benign and 42 were found to be malignant. All the malignant nodules were papillary thyroid carcinoma. The finding of this study showed 81.0% sensitivity, 63.6% specificity, 37.8% positive and 92.5% negative predictive value. An ECI value of 3.11 was used as a cut-off for diagnosing the malignant lesions by this technique (11). In the present study also a significant association was reported between the histopathological finding and ECI value. The present study showed that an ECI value of 4 can be used effectively to diagnose the malignant thyroid nodules.

Overall, the present study showed that USE can be effectively used to differentiate between the malignant and benign thyroid nodules and being a safe method this can be used widely. However, it should also be kept in mind that while investigating the nodules the distance from the carotid artery should also be considered as one of the limiting factors.

The present study also suffers from several limitations. Firstly, only one radiologist has evaluated the B-mode US images. Although the radiologist was quite experienced the interpretation might get biased because of this. Secondly, the cut-off value used in this study was inferred from small sample size and hence it might not prove to be the same in a large study population.

**Conclusion**

Overall, the present study showed that USE can be effectively used to differentiate between the malignant and benign thyroid nodules and being a safe method this can be used widely. However, the result of this study needs further validation with a larger study population.

**Conflict of Interest:** (Nil – There are “NO CONFLICT OF INTEREST”).

**Source of Funding:** By researcher (“HIM SELF”).

Ethical Clearance: Committee members are approved to perform a study about:

“The accuracy of Intrinsic Compression Ultrasound Elastography (E-Thyroid) in Differentiating Benign From Malignant Thyroid Nodule “

After discussion of study plan with researchers:

**References**

2. Lim DJ, Kim MH. Experiences of Intrinsic Compression Ultrasound Elastography (E-Thyroid™) in Differentiating Benign From Malignant Thyroid Nodule. 2015;


Comparison of Success Rate of Estrus and Pregnancy between Laser Puncture and Intra Vaginal Progesterone Sponge in Bali Cattle

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Abstract

The purpose of this study was to find a method of determining laser models and intra vaginal progesterone sponge in Bali Cattle. An assessment was carried out at Sapi Loka Grati, Pasuruan, Indonesia. The parameters observed included the symptoms of estrus, response of estrus, and the level of pregnancy. Twenty Balinese cows were divided at random in two groups. P1 treated with laser puncture at a certain point and P2 treated with 1.5 g of progesterone + 10 mg of estradiol benzoate intra vaginal sponge. The diagnosis of subsequent pregnancies was done by ultrasonographic examination on day 40th after artificial insemination (AI). The laser used in this study was a soft laser with power supply specifications as follows: 50 Hz, 220 Volts, 50 Watts, ranging from 0.2 Joules to 0.5 Joules. These lasers include semiconductor lasers and laser probes. Three acupuncture points were used (GV 4, BL-22, and GV-2). Furthermore, the intra vaginal progesterone sponge used was made from silicone plastic similar to the drugs containing medroxyprogesterone acetate. Data testing was done by using the T-test. Results demonstrated that the P1 responses of estrus appeared on day 3rd, while P2 on day 9th. There was no significant difference (p >0.05) in the accumulation of estrus and pregnancy. In conclusion, estrus synchronization and pregnancy rate could be efficiently achieved in Bali cattle using either laser puncture or intra vaginal progesterone sponge. However, laser puncture treatment induced more spread estrus with higher pregnancy rate.

Keywords: Bali cattle, estrus synchronization, intra vaginal progesterone, laser puncture, pregnancy.

Introduction

Bali cattle is one of the beef-producing livestock in Indonesia, but domestic Balinese beef production has not been able to cover the needs because of their population and low productivity levels. Various methods have been taken so that livestock productivity is maximized, including through improved management and quality of feed or by utilizing technology. The low population of Bali cattle is partly due the low level of reproduction¹. For better reductivity, estrus induction is one of important ways to be performed. Estrus induction is usually performed with hormonal method, such as application of PGF2α, but the disadvantage is relatively expensive².

Laser puncture technology has been proven to induce estrus with variable success in goats³,⁴, sheep⁴, and cows⁵. Irradiation of acupoints by means of low-level laser provides a relatively safe, noninvasive and efficient way of stimulation of acupoints⁶. Response and signs of estrus could apparently be improved after conducting a laser puncture procedure, so that could increase a pregnancy rate⁷,⁸. The application of laser puncture to Bali cows resulted in a response to estrus 90%, and from insemination, a pregnancy rate of 80% was obtained. The occurrence of conception in marriage indicates that estrus that occurs with laser puncture stimulation is followed by ovulation⁹. In this study, laser

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puncture has been reported to comprise a success rate of estrus and pregnancy with progesterone intra vaginal sponge in Bali cattle.

**Materials and Methods**

A total of 20 non-pregnancy Bali cows aged 2-3 years old, from cattle at Grati, Pasuruan, Indonesia were used in this study. Their body was scored at least 2 from 5 grades. All of the cows have been confirmed to have a normal estrus cycle that randomly divided into three groups. P1 was given a laser puncture and P2 was given progesterone (1.5 g) and estradiol benzoate (10 g) intra vaginal sponge.

![Figure 1. Acupuncture points related to reproduction](image)

P1 was treated using low-level laser puncture. The laser used in this study was a soft laser include semiconductor laser using power supply with specifications: 50 Hz; electricity of 220 Volts, 50 Watts output power, and laser probes. The laser puncture stimulation was applied on three reproductive acupoints (Figure 1): (a) GV 4, in the depression along the dorsal midline at the intervertebral space between L2 and L3 (for remove Qi/blood stagnation); (b) BL-22, at the first lumbar intervertebral space (L1-L2); and (c) GV-2, on the midline, in a depression at the first coccygeal vertebral space (C1-C2)\(^9\). The laser was applied to each acupoint 0.2 to 0.5 Joules performed sequentially for 3 min/day for 3 days. Observation the sign of estrus was carried out on day 3 and 4.

P2 was treated using intra vaginal sponge with plastic design (Pro Sponge progesterone) soft release containing progesterone for estrus induction in cattle. It was a large sponge capsule prototype and has a T-shaped plastic on top of it with nylon thread as a puller during extraction (length of ±15 cm and a width of 8 cm) on the caudal part. PGF2α recommended for use after 7 days stored and removed from the vagina on day 7 to ensure synchronized estrus\(^10\). The occurrence of estrus was observed and the animals which exhibited estrus were inseminated artificially with fresh sperm and the presence of pregnancy was noted. Examination to determine pregnancy was evaluated 40 days after artificial insemination (AI) using ultrasonographic examination. The data presented as means standard error of the mean and were compared using the Student’s T-Test. They were considered to be significantly different if \(p < 0.05\).

**Results**

**Sign of Estrus**

Physically, the occurrence of estrus was almost similar to the sign of estrus caused by natural mating, characterized by swelling of the vulva and reddish with transparent mucus accompanied by the appearance of the Bartholin gland. However, the color of vulva is redder with more transparent mucus than normal estrus that makes easier to detect.

**Estrus responses**

There was no significant difference \((p > 0.05)\) among treatment groups in the sign of estrus after treatment (Table I). All of the cows (100%) of the laser puncture and 90% of sponge intra vaginal progesterone had exhibited estrus. The interval of estrus was 3-6 days for laser puncture-treated cows and 9-10 days for progesterone intra vaginal sponge-treated cows, respectively.

Research result showed that from 10 cows treated by laser puncture which had estrus, there were 5 cows (50%) estrus on day 3; 3 cows (30%) estrus on day 4 and 2 others (20%) estrus on day 6. Whereas, from 9 cows treated by progesterone intra vaginal sponge which had estrus, there were 7 cows estrus on day 9, while 2 cows estrus on day 10. Laser puncture technology required interval 3 to 6 days after laser treatment and the most was 3 days. It means that laser puncture technology for estrus synchronization provides faster response compare to progesterone intra vaginal sponge.
The synchrony response of the cows in the present study was similar to that reported by others. Adikara (1995) obtained estrus success rate of 95% for treatment without seeing the estrus phase and 100% for treatment in the luteal phase. After the last 24 hours of shooting laser puncture, symptoms of estrus and hormonal examination showed an increase in reproductive hormones\textsuperscript{11}. Herdis (2011) demonstrated the estrus response to laser puncture treatment reached 100% when induced to luteal phase ewes, and 95% when induced to unknown estrous cycle with the means of estrus duration of 27 hours\textsuperscript{4}.

**Pregnancy Rate**

The results of the study did not show any significant differences with T-test ($p > 0.05$) in pregnancy rate (Table 1). By 40 days after artificial insemination, 100% and 90% of laser puncture-treated cows and progesterone intra vaginal sponge-treated cows were pregnant. These results indicate the pregnancy rate of estrus cows was very high, referring to the minimum standard of artificial insemination success according to the Directorate General of Animal Husbandry for an area in cattle is 55\%\textsuperscript{12,13}. Ultrasonographic examination appearing hyperechogenic embryo and hypoechochogenic uterine horn (Figure 2).

### Table 1. Different effect on laser puncture and sponge intra vaginal progesterone treatment on the number of animal estrus, interval of estrus, and pregnancy rate.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of cows</th>
<th>Percentages of estrus response</th>
<th>Interval of estrus after treatment (days)</th>
<th>% Pregnancy based on estrus response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laserpuncture</td>
<td>10</td>
<td>10 (100%)</td>
<td>3.7±0.5\textsuperscript{a}</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Sponge-progesterone</td>
<td>10</td>
<td>9 (90%)</td>
<td>9.2±1.2\textsuperscript{b}</td>
<td>9 (90%)</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b}Different superscripts at the same column indicate significant differences ($p < 0.05$)

Figure 2. Diagnosis of pregnancy appears on ultrasonographic examination showed hyperechogenic embryo and hypoechochogenic uterine horn
Discussion

The mechanism of stimulation of acupuncture points to the target organ is closely related to the acupuncture point as an active electric cell and acupuncture meridian. According to Adikara (2001), the framework of acupuncture points and acupuncture meridians is in accordance with the TAO philosophy which reveals the balance between living things and their environment. The theory that supports this is the existence of relationships between cells through cell bridges (intercellular bridges) that can be passed by material/protein with a certain molecular weight. Because the relationship is initiated by active cells, if there is a stimulus will cause a reaction of the emergence of cell activity in the form: changes in polarization, electrical charge, the occurrence of influx ions that stimulate rRNA and activation of mRNA to perform protein synthesis. The stimulus will be specifically communicated by other similar cells (active nature) so that it is an activity between the extra and intra-cells to the organ. Related organs will be stimulated to carry out a dynamic and physiological function with optimal capacity. The transfer of stimuli and matter through these specific cellular pathways is a dynamic energy change that leads to balance for the creature’s body\textsuperscript{14}.

The accuracy of the occurrence of estrus and pregnancy cannot be separated from the expertise of each researcher. Laser energy that falls on living tissue will give a biological reaction that depends on the type of tissue, the condition of the tissue and the amount of laser energy, it can provide destructive, inhibitory or stimulating effects\textsuperscript{15}. Meanwhile, the energy generated by He-Ne soft laser or low-level laser is a biostimulator effect that aims to stimulate estrus in livestock so that their reproductive functions can take place properly and normally. By using estrus synchronization (either laser puncture or sponge intravaginal progesterone) the cows will be bred within a short period of time, and the subsequent lambing will also take place over a short time period. This will allow producers to plan for labor input and feed resources\textsuperscript{16}. Another benefit of estrus synchronization is that it will allow producers to utilize other advanced breeding techniques such as artificial insemination. In cattle, estrus synchronization and artificial insemination (AI) can be used to maximize the reproductive potential of cows by incorporating superior genetics into their operations\textsuperscript{17,18}.

Conclusion

Based on the results, Bali cows can be synchronized using either of the two methods described, with no significant differences in the success rate of estrus and pregnancy rate. However, cows synchronized with laser puncture had a shorter interval to estrus than cows synchronized with sponge intravaginal progesterone.

Conflict of Interest: The author declare that they have no conflict of interest.

Source of Funding: We would like to thank Universitas Airlangga University via Post-Graduate Program and Research Bureau and Innovation F.Y. 2019.

Acknowledgements: We thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

References


ACO-based Type 2 Diabetes Detection using Artificial Neural Networks

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Abstract

**Background:** Type 2 diabetes is one of the most common diseases among people. Early diagnosis and treatment can reduce mortality and morbidity. So far, various solutions have been proposed to predict this type of disease.

**Materials and Method:** In this paper, a method for diagnosing diabetes was proposed using the Ant Colony Optimization (ACO) algorithm. To this end, data set properties are first reduced using artificial neural network features and then prepared for classification purpose. Finally, some components of accuracy assessment on the proposed system were calculated.

**Results:** The simulation results show that by adjusting the parameters of ANN and ACO, about 3.2% better prediction accuracy is obtained than other researches.

**Conclusion:** The results of experiments represent that the proposed method is proper for health management in diabetes.

**Keywords:** Diabetes detection, Artificial Neural Network (ANN), Ant Colony Optimization (ACO).

Introduction

Diabetes is a chronic disease that is diagnosed with high blood glucose levels. About half of diabetics have hereditary characteristics, which is one of the most important features of diabetes. Poor pancreatic insufficiency and insufficient use of insulin are the causes of diabetes. There are two major types of diabetes. Type 1 diabetes (T1DM) is when pancreatic secretions damage \( \beta \) cells and prevent a timely drop in blood glucose levels. Insulin resistance and inefficient insulin secretion are the causes of type 2 diabetes.

We need information technology-based methods to study high-risk groups at risk for diabetes. In this regard, meta-heuristic algorithms are a good tool that is used as a computational process to discover patterns in large data sets and includes several solutions such as evolutionary clustering, machine learning and neural networks.

Meta heuristic algorithms have been successfully used to solve various problems in various fields of basic sciences, engineering and even humanities. Weather forecasting, stock market analysis, system suggesting better customer management in banks and shopping malls, disease forecasting and medical data analysis are examples of applications of this category of algorithm. In more detail, extracting logical patterns from patients’ information in hospitals is essential for support as well as analysis, which requires the use of intelligent methods and data mining tools.

In recent years, various data mining methods have been used to predict diseases. Algorithms as well as various toolboxes have been developed and studied by researchers. Here are some examples of work done.

Patil proposed a hybrid predictive model that used the K-means clustering algorithm to validate the data class tag and the C4.5 decision tree algorithm to create the final model.\(^1\) The results of his proposed method have an accuracy of 92.38% in classification. Aliza compared the predictive accuracy of the multilayer perceptron (MLP) model in the neural network with the decision tree algorithms ID3 and J48.\(^2\) The comparisons showed the
superiority of the pruned J48 tree with 89.3% accuracy compared to the others with 81.9% accuracy. Codina proposed artificial flexibility on multilayer perceptron (AMMLP) as the final model for predicting diabetes with an accuracy of 89.93%. All of the studies used the Pima Indians Diabetes Database for experiments. Also, the toolbox used by most researchers to perform analyzes was WEKA software.

Research shows that in order to obtain results with higher accuracy, data preprocessing operations must be performed before applying the proposed solution to clear the data and make it more meaningful and logical. Vijoyan examined the benefits of using different data processing methods to predict diabetes. The preprocessing methods studied were principal component analysis (PCA) and discretization. Research has shown that preprocessing improves the accuracy of simple Bayesian classification and decision tree. This reduces the accuracy of the backup vector machine. He analyzed the high-risk indicators of type 2 diabetes using association rules and the evaluation of false positive rates. Zhou also suggested the area of the ROC curve, the values of sensitivity and specificity for validation and review of test results.

Sojania presented an Android-based application solution for raising awareness about diabetes in his article. The application uses the decision tree classifier to predict users’ blood sugar levels and provides information and suggestions about diabetes. The application uses data collected from a hospital in the Indian state of Chhattisgarh. Shi et al. have developed a model for assessing the risk of developing diabetes using a mobile device to prevent people from developing diabetes.

Some articles focus on improving the K-means clustering algorithm. For example, Wang proposed an improved k-means clustering algorithm by removing noise data. Sun proposed a solution to improve the selection of primary k-means clustering centers based on the Forubenius norm distance. Shoni Wang proposed an improved k-means clustering algorithm with variance in which the primary clustering centers were selected using the Hoffmann tree structure. Most articles improve the initial values of cluster centers.

People at risk for developing diabetes need to develop a set of rating standards for prediction. In this regard, Chandrakar and Saini presented the risk score of Indian overweight diabetes as a tool to show diabetes to solve the problem of diagnosis or late diagnosis of diabetes. Hamm and Lou proposed k-means clustering in pairs and limited to a certain size to represent the population at high risk of diabetes. This provided a tool for classifying the risk of the disease.

In summary, some of the research done to predict diabetes. However, the accuracy of the prediction and the validity of the data were not sufficient for real applications. In addition, most of the models presented by researchers work well only on specific datasets that do not have acceptable results on different datasets. Therefore, we need to create a new forecasting model with higher accuracy and compatibility with other data sets. In this paper, in addition to the Pima Indians dataset, two other datasets are used to test the proposed model.

Materials and Methods

In recent years, the use of data mining algorithms to predict diseases has increased. Some researchers have shown that it is possible to obtain predictive models from initial patient data. In particular, most of the published articles in the field of diabetes prediction have been aimed at improving the accuracy of the model. In this regard, some researchers have obtained good results using the WEKA toolbox on the Pima Indian dataset.

This section includes a review of the data set used for the experiment, the ant colony algorithm for data preprocessing, and an artificial neural network for data classification. All simulation and experimental processes have been performed using MATLAB 2018 software.

The Pima Indian Diabetes Database contains information on 768 patients living near Arizona. Tests performed with positive and negative results show whether the patient has diabetes or not. For all samples, 8 numerical properties are considered. This data includes data on a person’s health as well as the results of tests performed. The features in the dataset are as follows:

- **Number of pregnancies (preg)**
- **Plasma glucose concentration in 2 hours in a glucose tolerance test (plas)**
One of the most effective tasks in creating a model is data preprocessing, which plays an important role in the modeling process by increasing the quality of data in large quantities. At this stage, by using some appropriate methods, data set optimization is done. First, numerical properties that have a certain interval are transferred to the interval of zero and one and normalization is performed on them. In the second stage of preprocessing, outlier data is identified using ant colony optimization and the mean value is recorded instead. At this point, some unknown values recorded in the data set are also recorded with the mean value. Then in the next step, the degree of dependence of the properties on the class property is calculated and based on that, the less effective properties are excluded from the feature set. In this way, the complexity of the data is reduced.

• **Ant Colony Optimization Algorithm**

The ant colony optimization algorithm is a probabilistic technique for solving computational problems which can be reduced to find good paths through graphs. Artificial ants stand for multi-agent methods inspired by the behavior of real ones. The pheromone-based communication of ants is often the predominant paradigm used. Combinations of artificial ants and local search algorithms have become a proper method for numerous optimization tasks involving some sort of graph such as vehicle routing and internet routing.

In the real world, ants of some species wander randomly and upon finding food return to their colony while laying down pheromone trails. If other ants find such a path, they are likely not to keep travelling randomly, but instead to follow the trail, returning and reinforcing it if they eventually find food.\textsuperscript{15}

In this algorithms, an artificial ant is a simple computational agent that searches for good solutions to a given optimization problem. To apply this optimization algorithm, the optimization problem needs to be converted into the problem of finding the shortest path on a weighted graph. Initially, each ant stochastically constructs a solution, i.e. the order in which the edges in the graph should be followed. Secondly, the paths found by the different ants are compared. The last step consists of updating the pheromone levels on each edge.

• **Artificial Neural Network**

An Artificial Neural Network (ANN) is based on a collection of connected nodes called artificial neurons, which loosely model the neurons in a biological brain. All connection like the synapses in a biological brain can transmit a signal to other neurons. Artificial neurons that receive signals then process them and can signal neurons connected to them. The signal at a connection is a real number and the output of each neuron is computed by some nonlinear functions of the sum of its inputs.\textsuperscript{16} These connections are called edges. Neurons and edges usually have a weight that adjusts as learning proceeds. The weight increases or decreases the strength of the signal at connections. Neurons may have threshold points such that a signal is sent only if the aggregate signal crosses that threshold. Generally, neurons are aggregated into layers. Different layers may perform different transmissions on their inputs. Signals travel from the first layer to the last layer, likely after traversing the layers multiple times.

\begin{verbatim}
procedure ACO_MetaHeuristic is
  while not_termination do
    generateSolutions()
    daemonActions()
    pheromoneUpdate()
  repeat
end procedure
\end{verbatim}

**Figure 1. Pseudo-code for ACO.**\textsuperscript{15}

Results

To obtain the most accurate answer, 10-fold cross
validation and percentage split validation methods with different percentages were used. In the first validation method, the data set is divided into 10 subsets and in 10 consecutive periods, 9 subsets are used as training sets and another set is used for testing. In the second validation method, the data is used as a training set and the rest as a test set. Also found parameters are true positive rates (TPR), false positive (FPR), true negative (TNR), false negative (FNR), accuracy and f-measure.

In addition to the mentioned parameters, the ROC diagram related to the simulation is also calculated. This graph shows the ratio of positive rate to true positive false that the higher the level below the chart, the more accurate the model is.

The simulation results are shown on the dataset using each of the methods listed in Table 1. As we see, the application of the 10-fold method results in an accuracy of 99.24% and the use of the percentage split method results in an accuracy of 98.73%. If the accuracy of the model is considered as the main criterion of the accuracy of the proposed model, then the 10-fold validation method is superior to another. However, a closer look at the table shows that the amount of FPR in the second method is lower than in the first method, which means that in this method a person without diabetes is less likely to be labeled diabetic and in certain circumstances can it is a better option than the first method. Also, the true negative rate in the second method is superior to the first method (higher value), and this is useful when we want to identify people who do not have diabetes more accurately, in which case the percentage split validation method is better than the other. In other cases, the first method is superior.

Examining the table, it can be seen that in all accuracy parameters, the k-fold method is superior to the percentage split method; both in the parameters that have a positive aspect (such as true positive and false positive) or in negative parameters (true negative and false negative). The accuracy and f-measure values in

Figure 2. Flowchart for ANN
the first method are better than the second method.

Table 1. Simulation results on the dataset

<table>
<thead>
<tr>
<th>Method</th>
<th>TPR</th>
<th>TNR</th>
<th>FPR</th>
<th>FNR</th>
<th>Accuracy</th>
<th>F-measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-fold</td>
<td>0.995</td>
<td>0.984</td>
<td>0.052</td>
<td>0.076</td>
<td>99.24</td>
<td>0.989</td>
</tr>
<tr>
<td>Cross-validation</td>
<td>0.983</td>
<td>0.997</td>
<td>0.053</td>
<td>0.083</td>
<td>98.73</td>
<td>0.978</td>
</tr>
</tbody>
</table>

Figure 3. ROC on the dataset

**Conclusion**

As To prove that the proposed model improves the accuracy of the prediction, we compare the results with the experiments of other researchers in this field. Table 2 summarizes this comparison.

The accuracy obtained from the proposed method is 98.73% in the lowest case and 99.24% in the best case. As can be seen in Table 2, indicates the comparison of the accuracy of the proposed method with some recent approaches. Therefore, the proposed method is more appropriate than the other proposed methods.

The aim of this article was to create a suitable predictive model for diagnosing high-risk diabetes. In this paper, a new model for forecasting is proposed which includes two stages: data preprocessing phase and categorization phase. In the preprocessing phase, the ant data are identified using the ant colony algorithm and replaced with the mean value. The second phase is based on a neural network algorithm that uses which data is categorized. The results obtained from the simulation were compared with the results of other research works in this field and it was found that the accuracy of the proposed model is higher than other researches.
Table 2. Comparison of the proposed method with some other approaches

<table>
<thead>
<tr>
<th>Method</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed Approach</td>
<td>99.24%</td>
</tr>
<tr>
<td>Decision Tree</td>
<td>93.75%</td>
</tr>
<tr>
<td>Fuzzy</td>
<td>96.23%</td>
</tr>
<tr>
<td>Fuzzy + Neural Network</td>
<td>98.19%</td>
</tr>
<tr>
<td>ACO</td>
<td>95.83%</td>
</tr>
<tr>
<td>Bayesian Network</td>
<td>93.74%</td>
</tr>
<tr>
<td>Firefly + PSO</td>
<td>97.44%</td>
</tr>
</tbody>
</table>

**Ethical Clearance:** This article has been routed through the anti-plagiarism cell of Institutional Review Board.

**Conflict of Interest:** The author declares that they have no conflict of interests.

**Source of Findings:** Golestan University is the source finding of this paper.

**References**

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A Study for Assessment of Perception and Attitude of Medical Students about Medical Ethics, Consumer Protection Act (CPA) and its Influence on Violence against Doctors

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Abstract

Medical Profession was included under The Consumer Protection Act (CPA) in 1986 to provide a forum to safeguard the rights of the customers and establish guidelines for the speedy redressal of their grievances against unethical medical practices. This by far has been the most crucial legislations introduced in the framework of health professionals which over the years seems to have propelled them to practice in a guarded manner. In recent times this also seems to be flanked by a spurt in Violence against the Doctors enormously. It is thereby imperative for all medical professionals to not only have a comprehensive knowledge of ethics and laws related to health care and medicolegal issues but also its implications in their profession. In this very light the current study was conducted as an attempt to assess the perception of undergraduate medical students towards medical ethics, CPA and its influence on Violence against Doctors.

Key words: violence, influence, unethical, redressal, CPA.

Introduction

In the past few decades, bit by bit the trust which cultivated the doctor-patient relationship has started getting eroded and attained the shape of a crisis in the present century, where society does not hold this noble profession in high esteem.

In 1986, a comprehensive legislation Consumer Protection Act (CPA) was implemented in India to promote and safeguard the concerns of consumers. The CPA provides a forum to safeguard the rights of the customers and establishes guidelines for the speedy redressal of their grievances against unethical medical practices.

The health care providers were brought in the domain of a ‘service’ as defined in CPA by the Supreme Court decision in a case of Indian Medical Association (IMA) vs VP Shantha.¹

The CPA provides a forum to safeguard the rights of the customers and establishes guidelines for the speedy redressal of their grievances against unethical medical practices. Since ancient time; the relationship between doctor and patient was based on mutual trust and confidence. They were treated like God and their People revered and respected them. Today, the fast pace of commercialization and globalization affect all spheres of life including medical profession.

With inclusion of medical profession under CPA, a surge in litigations arising out of breach in medical/dental profession is seen.

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Not only that it also forms a background for violence against doctors, which is quite prevalent in Asian countries like India, Pakistan, Bangladesh, Israel, and China. Further, the prevalence rates have been higher in comparison to Western countries. The IMA suggests that up to 75% of doctors have faced some kind of violence at work, which is similar to the rates from other countries in the continent. This may be in the form of threatening calls, verbal abuse, intimidation, damage to the property, physical assaults causing injuries (simple or grievous), murder, and arson.

Medical professionals who faced violence have been known to develop psychological issues such as depression, insomnia, posttraumatic stress, fear, and anxiety, leading to absenteeism. Many have lost their clinics, injured themselves, lost lives, and also tarnished their reputation as a professional due to these incidents.

It is the need of the hour for us to address the issue of surging Violence against Doctors and recognize the causes where it stems from. In this background this study has been observed to discern the influence of CPA on increasing Violence against medical professionals.

**Material & Method**

A cross-sectional survey was conducted in the medical educational institute.

**Study Population**

189 MBBS students studying in 2nd & 3rd Professional at Rama Medical University Kanpur (U.P.) India; were included in present study.

**Ethical Clearance and official permission**

Before starting, ethical approval was obtained from the Institutional ethical committee and official permission was taken from the concerned authorities.

**Pre-testing of Questionnaire**

Prior to the data collection the questions were pre-tested among a group of professionals in order to ensure the level of validity and degree of repeatability.

**Questionnaire Design**

A self-administered, structured questionnaire written in English validated through a pretested survey including 16 items was used to assess the perception of undergraduate medical students towards medical ethics, CPA and its influence on Violence against Doctors. The questions were aimed to ascertain perspectives of participants towards medical ethics, CPA and its influence on Violence against Doctors.

The purpose and details of the study was explained to all the study participants. They were exposed to closed ended pre-tested and pre-validated questionnaire regarding awareness of Consumer Protection Act after obtaining their informed verbal consent. A self administered questionnaire having 16 questions related to various aspects of CPA and violence against doctor, was provided to the participants. Confidentiality was maintained.

The study participants were given the questionnaire (with complete explanation on how to fill in the questionnaire) on the day of visit by a single investigator. The participants were asked to respond to each item according to the response format provided in 30 minutes time. The questionnaire was later checked by the investigator. Data obtained was analyzed using SPSS version 20.

**Observation & Results**

A total of 189 students (81 Male and 108 Females) were considered for present study. Among 189 respondents, 82 were 2nd Professional and 107 were from 3rd Professional students. Most students were well aware of CPA. Out of 189 participants, 85% were well aware of Consumer Protection Act.
Table No. 1 Perspectives & Attitude towards CPA, rise in Medical Negligence and its influence on Violence against Medical Professionals

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reasons Identified</th>
<th>Agree</th>
<th>Disagree</th>
<th>Neutral/ Don’t know</th>
<th>Total Agree(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lack of Competency</td>
<td>Male:13 Female:23 Total:36</td>
<td>Male:44 Female:70 Total:114</td>
<td>Male:32 Female:15 Total:47</td>
<td>19</td>
</tr>
<tr>
<td>5.</td>
<td>Apathetic attitude of Healthcare Professionals</td>
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<td>Male:69 Female:81 Total:150</td>
<td>Male:01 Female:02 Total:03</td>
<td>19</td>
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<td>6.</td>
<td>Compelling workload</td>
<td>Male:57 Female:75 Total:132</td>
<td>Male:09 Female:12 Total:21</td>
<td>Male:12 Female:24 Total:36</td>
<td>70</td>
</tr>
<tr>
<td>9.</td>
<td>Do you think law to protect medical professionals needs to be stringent</td>
<td>Male:75 Female:91 Total:166</td>
<td>Male:03 Female:10 Total:13</td>
<td>Male:06 Female:04 Total:10</td>
<td>88</td>
</tr>
</tbody>
</table>

Discussion
Among 189 participants; 161 (85 %) attributed fear of CPA and litigations against the doctors as a cause of upsurge in violence against the health care professionals. With the advent of Consumer Protection Act, 1986 creating consumer disputes redressal agencies (C.D.R.As) there were drastic changes. This was immediately resented by the medical community who raised their shields and challenged the applicability of the Act to them. There is a considerable scope for studies to explore the role of unsatisfied doctors vis-à-vis their
services now being under the ambit of CPA.

As part of the medical curriculum, all doctors are taught clinical behavior but not all are taught empathy. Whereas in clinical practice, effective patient–doctor communication involving receiving an explanation for the occurrence of the symptom/sign, likely duration of treatment, the lack of unmet expectations, and empathy are associated with overall patient satisfaction with the services. In our study more than 80% students admitted lack of training and communication skills as a causative factor.

Such violence may end up with a variety of psychological and psychiatric conditions like insomnia, depression, Post-traumatic Stress Disorder (PTSD), agoraphobia, fear and anxiety, often leading to acute absenteeism from work and loss of productivity in the long-term.

Many a time, the patient does not comprehend the gravity of the situation and expects a better chance of complete recovery due to improper explanation by the treating doctor. In a country like India, due to the scarcity of doctors and health-care facilities, these issues are seldom given importance, which makes this one of the important causes of rising violence against health-care practitioners in the country. Statistics from a recent Indian study of 151 doctors, evaluating workplace violence, suggested that only six of them had received some formal training in effective communication and five of these doctors belonged to psychiatry department where it is a part of the curriculum. This suggests that there is an urgent need for improving the communication between the patient and doctor by imparting training to the current generation of doctors.

In India, a single doctor caters a population of 1,445 which is much lower than the WHO’s standard of one doctor for 1,000 people. This leads to a heavy workload with meager facilities. The study shows that 92% students accounted compelling workload as one of the contributory factors towards rise in violence.

71% students feel the need for more stringent laws to prevent violence against the doctors. Having a Central law for prevention of violence against healthcare persons and institutions would also help but a change in the IPC to make such violence a cognizable offence with stringent punishment is the need of the hour.

Limitations:

Being a cross-sectional study with relatively small sample size it is difficult to generalize these findings for the entire nation. Further, it was a questionnaire study, knowledge and awareness of participants may or may not be predicted, reflecting the intrinsic limitations of such studies. Further studies should be conducted with applicability of some better tools.

Preventive measures and Recommendation:

1. Well trained security staff must respond immediately in case violence occurs and assist if needed.
2. All medical centers must have an advance and robust public address system with distinct alert siren to cope up with such unfortunate incidences.
3. All the available staff should proceed to support the professionals under threat with a calm attitude and avoid any argument which may further worsen the situation.
4. Few unconcerned senior staff members, may try to talk with the patient’s relatives and try de-escalating the situation.
5. The practice of this drill should be done regularly in every health care establishment.

In addition, all hospitals should have a good network of closed circuit televisons and have a zero tolerance to workplace violence. Such steps have been taken already in developed countries.

Conclusion

Public awareness regarding medical negligence and patients right is growing worldwide including India. CPA is a reality which doctors needs to be trained exclusively.

Violence should be condemned in any form. However, against health care worker (HCWs) it is indefensible and should be dealt with stern efforts. Doctors and patients need to develop a better understanding between each other. Both have an important role to play in avoiding unnecessary violence. HCWs, while focusing on treatment, should not forget to communicate with the patients about the progress of therapy. A new chapter introduced in the medical curriculum focusing on the medical ethics, medicolegal knowledge and other nuances.
Patients, on the other should realize that medicine is not magic and a doctor is not God. It is to be remembered that the fight is against diseases and not against doctors.

These measures would definitely address the problem of violence against healthcare service professionals and damage to the property of clinical establishments across the country. Stronger political commitment to safeguard medical professionals by framing strict central laws and implementing them at the ground level would go a long way in resolving the long overlooked issues of violence against doctors.

**Conflict of Interest:** None

**Ethical Clearance:** Taken from the Ethical Committee of the Institute

**Financial Assistance:** Nil

**References**


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To Study the Intervention of Bell’s Palsy by Video Self Modelling through Kinect Azure: A Research Protocol

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Abstract

Background: Bell’s palsy is the common acute mono-neuropathy, and is commonly associated with facial paralysis or weakness of facial nerve. It is unilateral facial nerve paresis or paralysis. The cause of Bell’s palsy is suspected to be herpes simplex virus infection of nerve. The nerve get swollen because of this viral infection and is compressed in its canal as it passes through the temporal bone. Grading systems for the assessment of movements and asymmetry of face in Facial palsy are divided into computer-based and traditional grading systems. The program that uses Kinect Azure provides assessment method for evaluation of asymmetry of face at rest and the rating of facial palsy during voluntary activity of various areas over face. This study aims to investigate the intervention of facial palsy by video self modelling with the use of Kinect Azure.

Methods: 20 participants will be selected. Each group will include 10 subjects. Group A will receive conventional treatment, electrical muscle stimulator (EMS), and visual feedback.

Group B (Experimental group) will receive conventional treatment, electrical muscle stimulator and video self modelling. Each participant would be presented with their own videotape of video self-modeling, which included the best attempts at their evenest acts (smiles). Following 2 weeks of tape viewing the actions will be assessed. The outcome of the treatment will be assessed by Kinect Azure.

Discussion: Traditional methods for documentation of treatment effect have been through scales and questionnaires which at times are little complex and also difficult for patients to interpret. Hence this experimental and comparative study aims at focusing on the effective use of Kinect to document outcome for bell’s palsy.

Key words: Bell’s palsy, physical therapy, Kinect.

Introduction

Bell’s palsy is the common acute mono-neuropathy, and is commonly associated with facial paralysis or weakness of facial nerve. It is unilateral facial nerve paresis or paralysis. This disorder causes complete or partial inability of the paralysed side of face to voluntarily move facial muscles. The paresis or paralysis of face in Bell’s palsy result in inability to close the eyelid and temporary oral incompetence resulting in possible injury to the eye. Annual incidence of palsy is 15 to 30 per 100,000 people, with equal numbers of women and male affected. Any side of the face has no predilection. The palsy of Bell was identified in patients of all ages, with peak incidence in the 40s. It occurs more frequently in diabetes patients and pregnant females.

The cause of Bell’s palsy is suspected to be herpes simplex virus infection of nerve. The nerve get swollen because of this viral infection and is compressed in its canal as it passes through the temporal bone. Symptoms typically begin in the first week, and then gradually resolve over 3 to 3 months. It is in patients with diabetes, and while it can affect people of any age, incidence peaks in 40s. Paresis is the most disturbing symptom of Bell’s palsy; up to three quarters of affected people assume they have had a stroke or an intracranial tumour. The palsy frequently starts unexpectedly and...
progresses quickly, with maximum facial weakness occurring within two days. Hyperacusis, reduced tear production, and altered taste may be associated with symptoms. Patients may also report otalgia or aural fullness, and facial or retroauricular pain, usually mild and preceding palsy. Severe pain indicates herpes zoster virus, and can lead to a vesicular eruption and progression to Ramsay Hunt syndrome. Features can lead to a mild polynuropathy. A gradual progressive paralysis with other cranial nerve defects or headache increase the neoplasm possibility (4).

Video self-modelling is used with movements of face affected by facial nerve palsy (Lower Motor Neuron type). Although self-modeling patients view video clips of themselves engaging only in the correct, adaptive behavior and so the treatment relies on the patient being able to generate the desired form of behavior at least once. The action, or movement pattern, is recorded, edited into a short set of images by only choosing the best presentation and then given to the patient to watch again before replaying. Analyzing one’s best attempts at a desired reaction has been used to promote motor learning in a variety of therapeutic settings, and it improves the degree of success that integrates previously unachievable abilities. vBecause an successful smile provides the audience with a powerful emotional input, this study was designed to examine the use of video self-modeling as a method of adapting the smiles after facial nerve palsy.(5)

Evaluation of paralysis of face and quantitative grading of asymmetry is important to measure the severity of the disorder as well as to monitor its progression or improvement. As such, a precise quantitative grading system is needed which is easily understand, cheap and has minimum variability. As there is clearly a need for a clinically feasible tool that can assess the severity of the disease and the resultant loss of function from Facial palsy and can also calculate the efficacy of medical care or surgery. Such a tool should be qualitative, standardized, depending little or no on the observer, and cost-efficient.

Grading systems can be divided into conventional and computer based grading systems for determining facial gestures and facial asymmetry in facial palsy.(6) Modern approaches include the House-Brackmann grading system (HBGS)-The House and Brackmann grading system is recommended as a common standard for determining the degree of facial paralysis and is a clear and accurate method for evaluating facial function..(7) Functional disability index – It is a disease-specific, self-reporting functional status instrument that provides an essential component for the evaluation of citizens with facial neuromuscular disorders.(8) Many programs include Burres-Fisch, Nottingham, Sunnybrook, and many others. Computer-based FP grading systems including video recording and image processing are also proposed. (6)

Kinect Azure provides assessment method for evaluation of asymmetry of face at rest and the rating of facial palsy during voluntary activity of various areas over face. This study aims to investigate the intervention of facial palsy by video self modelling with the use of Kinect Azure.

Aim:

This study aims to analyze the intervention of Bell’s palsy by visual self-modeling using Kinect Azure.

Methodology

Study setting:

The trial will be carried out in HumEn research lab and Neuro Physiotherapy Department of Ravi Nair Physiotherapy College, DMIMS, Sawangi(Meghe), Wardha, Maharashtra, India ,after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences,Deemed to be University.

Study Design and Sample Size:

The design of the study is a single blinded randomized controlled trial of a Kinect Azure for individual diagnosed with bell’s palsy and it is an experimental and comparative study.

By using purposive sampling we will select 20 subjects(n=20) and will include 10 subjects in each groups(Group A and B). All topics will be clarified in detail about the research and a written informed consent will be taken. Group A will receive conventional treatment, electrical muscle stimulator(EMS),and visual feedback.Group B(Experimental group) will receive conventional treatment, electrical muscle stimulator and video self modelling. All topics will be clarified in detail about the research and a written informed consent will be taken. The schedule of enrollment, interventions, and assessments of the study is illustrated in Figure 1.
### Figure 1 Schedule of enrolment, interventions and assessments.

<table>
<thead>
<tr>
<th>TIMEPOINT</th>
<th>STUDY PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enrolment</td>
</tr>
<tr>
<td>ENROLLMENT:</td>
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<tr>
<td>Eligibility screen</td>
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<tr>
<td>Informed consent</td>
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<td>Allocation</td>
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<tr>
<td>INTERVENTIONS:</td>
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<tr>
<td>{Conventional}</td>
<td>x</td>
</tr>
<tr>
<td>{video self modelling}</td>
<td>x</td>
</tr>
<tr>
<td>ASSESMNTS: House-Brackmann Scale, Facial disability index</td>
<td>x</td>
</tr>
<tr>
<td>Outcomes measure: Kinect Azure</td>
<td>X</td>
</tr>
</tbody>
</table>

Participants

The Inclusion Criteria for the participants are as under:

1. Those who are willing to participate.
2. Patient affected by acute onset paralysis without detectable cause
3. Those with Idiopathic facial paralysis
4. Rehabilitation treatment carried out at our own hospital
5. Those who have unilateral facial paralysis-LMN type
6. Those who are between 20 to 42 years of age.
7. Those who scored above grade 3 on House brachman scale
8. Those have Normal superficial and deep sensation

The Exclusion Criteria for the participants are as under:
1. Those who are not willing to participate
2. Those with any type of facial fracture
3. Those with Known traumatic, inflammatory, Neoplastic pathology of facial nerve
4. Those with Bilateral facial paralysis
5. Those with UMN type facial palsy
6. Those who have Disease of central or peripheral nervous system
7. Those who scored below grade 3 on House brachman scale.
8. Those with Recent head injury
9. Those who have Psychiatric disease

PARTICIPANT TIMELINE:

The study duration is of 6 months and intervention duration is 2 weeks.

Assessment will be done on 1st day of visit following 2 weeks of tape viewing the actions will be reassessed.

RECRUITMENT: The neurologists and health care practitioners working under DMIMSU are invited to refer the prospective patients to our inpatient department (IPD). Regular visit to Neuromedicine, Neurosurgery wards will be done and contact will be maintain with doctors, record maintaining office for cases that will enrolled in hospital so that can be taken for study. The patients who are already undergoing treatment in our IPD and diagnosed with facial palsy will be assessed for the eligibility in the study as per the inclusion and exclusion criteria. Informed patient consent will be taken before allocation and after elaborating the purpose, nature, procedure, benefits and effects of the intervention.

Implementation:

Selection of the participants will be supervised by the research coordinator and principal investigators.

Blinding:

Tester(s) will be blinded to assign the subjects to the group. To ensure blinding, subjects will be mandated not to reveal any details of their treatment to the tester.

Study procedure:

The participants will be categorized into 2 groups:

Group A: (Conventional physiotherapy): The participants in this group will undergo 1 hour of conventional physiotherapy program daily, 5 days per week for 2 weeks. It will be performed by a physiotherapist in IPD. It will comprise of electrical muscle stimulator, visual feedback, facial exercises.

Group B: (Video self modelling combined with conventional physiotherapy): The participants in this group will undergo 30 minutes of conventional physiotherapy and 30 min of video self modelling based physiotherapy daily for 5 days per week for 2 weeks provided by physiotherapist in IPD. Each participant would be presented with their own videotape of video self-modeling, which included the best attempts at their evenest acts (smiles). In the following way we will make a videotape. A mobile video camera will be at a constant distance from the subject matter and set to run at session start. Subjects would be asked to complete a series of 5 smiles that included both their normal “everyday” (nonlinear) smile and their best (linear) “adapted” smile. The entire tape will be reviewed after a session, and 2 or 3 of the best smiles will be recorded using video editing software. Following 2 weeks of tape viewing the actions will be assessed.

The outcome of the treatment will be assessed by Kinect Azure from first day and after 2 weeks of intervention.

OUTCOMES

Primary outcome measures:

1) House-Brackmann Scale- For assessment of degree of facial paralysis.

2) Facial Disability Index used as an initial assessment tool and as a monitoring instrument to view
the outcome of intervention.

**Secondary outcome measure:**

Kinect Azure

**DATA COLLECTION AND MANAGEMENT**

**Data collection**

The assessment data will be collected from a pre-established spreadsheet with the baseline characteristics variable. Testing data will be put into a secure REDCap database. The nonelectronic data, such as hard copies of assessment forms, signed consent forms, etc., will be stored securely in the study setting. The employment of regular feedback concerning adherence and reminder phone calls (for attending the treatment) will be done.

**Data management:**

Data collection and documentation will be done under the guidance of the principal investigators. The study documentation will be evaluated thoroughly for accuracy. The Excel spreadsheet will be released at the end of the study to an allocation blinded statistician for conducting the necessary analysis, following which unblinding of the groups will be done. Checklists are used to prevent missing data due to the improper staff procedure.

**Statistical Analysis Plan**

Therapy induced changes in the primary outcome measures will be analysed via mixed-effects linear models across ‘time’ (pre-intervention vs post-intervention) and ‘group’ (Experimental vs control). The comparison will be done between the two groups using t-tests for the demographic measures and initial scores on outcome measures. For the interpretation of the results, we will set significant differences. Significance will be set at P less than 0.05. The results will be accounted for as per the CONSORT guidelines.

**BIAS**

Our study will have a low degree of selection bias (Oculus Quest). Measures will be taken to prevent attrition bias by giving reminder calls before each intervention and by giving transportation aids to those who require it. Thus, we anticipate a low percentage of dropouts.

**Discussion**

Our study aims to estimate efficacy of video self-modelling compared to conventional physiotherapy in individuals with Bell’s palsy. Physical therapy has played a significant part in Bell’s palsy management. Rehabilitation appeared effective in recovering facial symmetry, reducing paresis severity by 0.6 grades on the HB scale, and controlling synkinesia. Physiotherapy rehabilitation of an adapted (more symmetrical) smile was investigated by Dr Susan E in FNP subjects 1 year after the start, using video self-modeling (video replay of only the best adapted smiles) and implementing intentions (preplanning adapted smiles for specific situations and concluding that reaction time (RT) for the initiation of adapted smiles was 224 ms faster, adapted smiles were completed 544 ms faster, adapted smiles had higher overall quality, movement control and symmetry ratings, and Facial Disability Index scores also improved. His study supports these techniques of rehabilitation to maximize the quality of the smiles following facial nerve palsy. Kinect platform can be used to develop low cost approaches to measure the movement aspects objectively.

Research and development prospects and future therapeutic applications work with the Kinect are comprehensive. This will help to make diagnostic and prognostic evaluations.

**Ethical Approval and Dissemination**

Ethical approval will be taken from institutional ethical committee. The DMIMS which will fund research and the subjects which will participate in the study will be able to access the research’s main findings. For the enrolled subjects, data will be held safely for a minimum of five years. Once data collection is complete, a completion report will be produced for statistical analysis and sent for publication after review by institutional research cell.

**Patient Consent**

Principal Investigators will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality.

**Confidentiality**

The study program will be explained to the participant and one of his/her relative, and the principal investigator will take personal information. The consent...
form will include the confidentiality statement and signatures of the principal investigator, patient and 2 witnesses. If required to disclose some information for the study, consent will be taken from the patient with complete assurance of his confidentiality.

**Author’s contribution**

DJ suggested the design of the study. MD and DJ led to the creation and design of the study. MD wrote the manuscript of this article. MD and DJ read and approved the final manuscript for publication.

**Declaration of interests:** The authors declare no conflicting interest.

**Funding:** No direct support will be taken for funding this research from any public and private organizations. The Department of Physiotherapy under Datta Meghe Institute Of Medical Sciences, Deemed to be University, will provide the necessary material for the research.

**Reference:**

Impact of Bedaquiline on Multidrug-Resistant Tuberculosis Treatment to Mother and Baby: An Incidental Case

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Abstract

Background: Bedaquiline is a new drug which is recommended by World Health Organization (WHO) for individual regimen drug resistant-tuberculosis (DR-TB). The presence of regimen DR-TB in the blood is long enough with T¹/² 5-6 months, that is why bedaquiline is given on six months. Bedaquiline is not recommended for pregnant women because there is no data related to safety. The author will report an incidental pregnant female on bedaquiline treatment.

Case presentation: A 24-year-old woman with multidrug resistant tuberculosis (MDR TB) on individual regimen bedaquiline had incidental pregnancy at the 6th month treatment. In the beginning, the patient used bedaquiline because of the intolerance of second-line injectable drug. Bedaquiline regiment was used for 24th week. Pregnancy occured at the 6th month treatment. The patient continued the pregnancy and the MDR TB treatment was continued without bedaquiline. Nausea and vomiting were getting worse. Preterm labour occured in 33/34 weeks by cesarean section. The baby had severe asphyxia, used continuous positive airway pressure (CPAP), treated in NICU, had low weight but there was no disability. After several days of treatment, the condition of baby was improving and was able to outpatient.

Conclusion: The preterm labour, low birth weight, and neonatal emergency occured in a pregnant woman with MDR TB on individual regimen bedaquiline. Mother and baby can survive. More case and research data are needed on the safety of bedaquiline during pregnancy.

Keywords: bedaquiline, multidrug resistant tuberculosis, pregnancy, mother, baby

Introduction

Treatment of multidrug resistant tuberculosis (MDR TB) is increasingly complicated when the patient is pregnant. Pregnancy can occur when a patient has been diagnosed with MDR TB and is undergoing treatment or the pregnant woman diagnosed with MDR TB. There are still some controversial opinions regarding the management of MDR TB with pregnancy (¹). Some clinicians recommend terminating pregnancies and continuing MDR treatment. Other clinicians delay administration of the drug temporarily, especially in the early trimester. This doubt occurs because there is no data on the safety of MDR TB drugs in pregnant women and fetuses.

Case Presentation

A 24-year-old woman with MDR TB on individual regimen bedaquiline had incidental pregnancy at
the 6th month treatment. In the beginning, Acid-Fast Bacillus (AFB) sputum was positive, GeneXpert was *Mycobacterium tuberculosis* detected very low, Rifampicin Resistance detected, drug susceptibility testing (DST) was resistant of Rifampicin, Isoniazid, Ethambutol, Streptomycin, and sensitive of Kanamycin, Ofloxacin, Amikacin. The patient had moderate depressive episodes without psychotic symptoms because she had received TB treatment for the third time and treatment of MDR TB which took longer. Electrocardiography was within normal limit and there was no elongation of corrected QT interval (477 ms QTc). There was mild hearing loss on the right ear based. The laboratory data was normal. The patient had a strong motivation for treatment and recovery.

**Before Pregnant:** The patient used individual regimen bedaquiline because of intolerance of second-line injection drugs. Bedaquiline regimen was used for 24th week. The regimen was Pyrazinamida 1000 mg / Levofloxacin 750 mg / Ethionamide 500 mg / Cycloserine 500mg / Para-amino salicylic acid 8 mg / B6 100 mg with Bedaquiline 400 mg every day in the initial 2 weeks, and then continued with bedaquiline 200 mg three times a week. The patient had nausea and vomiting but medication was taken regularly.

**Pregnant:** Pregnancy occurred at the 6th month treatment. The patient continued the pregnancy and MDR treatment was continued without bedaquiline. The regimen was Pyrazinamida 1000 mg / Levofloxacin 750 mg / Ethionamide 500 mg / Cycloserine 500 mg / Para-amino salicylic acid 8 mg / B6 100 mg. Nausea and vomiting were getting worse and she had lost 5 kg of weight the first month of pregnancy. The patient had routine control of the pregnancy. There were no serious complaints during Antenatal Care.

**Labour:** Preterm labour occurred in 33/34 weeks gestation. Pregnancy had been maintained by tocolytic but it was unsuccessful. The bullae in both lung fields patient should the patients had sectio caesaria surgery and cannot vaginam labour (figure 1). There was no complication such as bleeding on the mother during operation process. The baby was female with clear coloured membranes but the baby had severe asphyxia (Apgar Score 3) in the first minute and was getting increase at the next evaluation. The baby used continuous positive airway pressure (CPAP) and treated in Neonatal Intensive Care Unit (NICU) for several days. Her weight was 1,950 gram so classified as low birth weight (LBW) but there was no disability. Babygram was within normal limit (figure 2). After several days of treatment, the baby was getting improved and transferred to the baby care room, which was then allowed to outpatient.

![Figure 1. Chest X-ray revealed the fibrotic process and multiple bullae in right and left apex hemithorax.](image1)

![Figure 2. Babygram.](image2)
Discussions

Pregnant women with untreated TB have morbidity, the risk of vertical transmission, and mortality rate of 40% (2). There is an increased risk of pregnancy complications such as spontaneous abortion, oligohydramnios, preterm labour, Intra Uterine Growth Restriction (IUGR), Intrauterine fetal Death (IUFD), and an increased risk of neonatal death. Therefore, pregnant women with TB need to be treated effectively before giving birth, including women with MDR TB. However, the clinician is often doubtful about the treatment because there is no data on safety.

Bedaquiline is bactericidal which is given an additional drug for MDR TB patients. Although bedaquiline is not teratogenic, there is no data on the safety of pregnant women. In this patient, pregnancy occurs at 6th month bedaquiline treatment. Bedaquiline has already stopped but the half-life (T1/2) is still around 4-5 months (0, 4). However, Bedaquiline is still in the blood and passes through the placenta to the fetus. This can impact the pregnancy in the first and second trimester.

Although bedaquiline was declared not teratogenic and mutagenic in the in vivo and in vitro studies, there was no study in pregnant women (1, 5, 6). In this patient, Partus Premature Imminent (PPI) occurs at 33/34 weeks of gestation by cesarean section. The baby had severe asphyxia with Apgar Score 3, but with good treatment, the Apgar score was increased in the next evaluation. The baby used CPAP and treated in NICU. Baby had low weight but there was no disability. After several days of treatment, the baby condition was improving and was able to outpatient.

Transmission of TB from mother to baby can occur during the fetus or after childbirth. TB in the fetus can occur when there is spread through the placenta during pregnancy. But this congenital tuberculosis is rare. In this situation, Mycobacterium tuberculosis has been identified in the amnion, decidua, and chorionic villi (7, 8). But this rarely happens if the mother has undergone effective treatment in pregnancy. In this patient, there is no specific process in umbilical histology examination. This is in accordance with the theory because mothers have received TB treatment well.

Conclusion

The preterm labour, low birth weight, and neonatal emergency occur in a pregnant woman with MDR TB on individual regiment bedaquiline. Mother and baby can survive. However, further monitoring is needed regarding the possibility of transmission of TB after birth. More case and research data are also needed on the safety of bedaquiline during pregnancy.

Ethics Statement

All procedures performed in studies / case report were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The authors explains the aimed, benefits, and rights of the participant during the process of collecting data to the patient’s guardian, if the participant agrees we ask the participant to fill out an informed consent sheet.

Conflict of Interest: The authors report no conflict of interest in this publish.

Acknowledgement : We would like to express our sincere thanks to the Indonesia Tuberculosis International Meeting (INATIME) event which facilitated us to present this research on 5-7 April 2019 at Surabaya, Indonesia.

Funding: None

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Migraine Headache and Gender Differences of Bagdad City Population/ Iraq

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Abstract

Migraine headache is a very common neurobiological disorder that is caused by increased excitability of the CNS. It has been increased the diagnosis of migraine headache which is based on the headache's characteristics and associated symptoms. It disturbs patients' quality of life and weakens their work, social activities, and life quality. Recently WHO organization classified migraine headache as 19th among all the disabling diseases. Data on sex related differences in migraine headache more marked overlapping with migraine features in females' subject. We have conducted survey on gender difference and prevalence of migraine having the sample size of 500 patients from Iraq.

We Selected Of 228 patients from 7-47 years who diagnosed with migraine headache according to Bagdad General hospital record from December 2018 to September 2019. After consent form signed, subjects were requested to fill questionnaire included demographic, clinical characteristics, history of sickness, duration of it, time happened in the months, general health, and other questions

The primary outcome was women are more prone to migraine attacks than men. 2-4 hours of pain was the most common period of headache along with triggering factor of stress and poor sleep. Migraine headache is a chronic daily sickness which is severe, and major health problem and disorder that effect on life quality.

in summary, we would confirm that there is presence of distinct gender related differences in migraine headaches add to some novel information that increase of level of migraine associated with family history.

Key Words: Migraine, Headache, gender- difference, disorders.

Introduction

Migraine headache are now acknowledged as one of the most prevalent cause of public ill health that would be affecting people health in all countries. This awareness comes, in the principal, from the multiple repetitions of the several of Global Burden of Disease (GBD) study since the year 2000 to 2018(1). one of GBD studies conducted in 2012 found tension and migraine types headache would be 2nd and 3rd the most prevalent disorders in the world (only dental caries was more common)(2).

Recently, global burden of disease increased and well cognizant during these years. Particularly by the population-based studies conducted in countries all over the world by what has been known as Lifting The Burden (LTB) (3, 4). Moreover, although LTB’s investigated all the use similar, identical methodology established for the purpose headache happening (5).

it has been defended several types of migraine, two principal types are: migraine with aura which is recognize and known as classic migraine and migraine without aura that are common migraine in many countries. The most common sort of migraine symptoms involved transient visual, sensory turbulences, motor interruption or language instabilities that are remain for 15 m or more than an hour and this mostly happened before onset of headache. while migraine Without aura

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E-mail: wael.walid@ruc.edu.iq
signs continuous from 4 to 72 hours and it symptoms are included sensitivity to light, nausea and vomiting, ailments in sound, touch, odor\(^6\).

Migraine is categorized as one-sided headache. The pain is location mostly behind eye, neck, face and shoulder\(^7\). Migraine confrontations usually are divided into five phases including by warning phase that are definite by physical and mental changes which trimmings after 1 to 24 h. second phase known as Aura phase which may include changes in visual (flashing lights sensitivity. its finales after 5 m to an hour. third phase would be Headache. it includes head pain usually experiences unilateral pain and some patients specific have bilateral pain. This particular phase symptoms may be involved nausea and vomiting, photophobia or phonophobia that take at least 4 hours or 3 days. forth phase known as resolution phase which is described as sufferer pain ended slowly. Sleeping is sufficient to cure this attack. Last phase would be postdromes or recovery phase. In this phase the discomfort and pain experiences are similar to symptoms happened in the first phase\(^8, 9\). Migraine can usually occur from environmental and genetic factors. Food is the most triggering factor other than fluctuation hormonal levels. It is most commonly occurs between the age of 20 to 50 years. It affects more boys than girls before puberty but often 2 -3 times more in elderly women than men. After menopause, there is a considerable reduction in migraine\(^10\). Many causes can lead to migraine such as environmental and genetic factors. Many studies support that Food is the greatest causing factor other than fluctuation hormonal levels\(^11\).

In the current study we aimed to describe the 1-year prevalence of these headache disorders in Iraq adult population, and present analyses of associations with demographic variables and disorders and history.

**Material and Methods**

In the medial of Iraq particularly in Baghdad city and from Number 2018 to Number 2019. After consent form has been singed responses to a feedback questionnaire were obtained from 330 individuals (238 females & 92 Males) from patients who attended headache and neurology disorders center, the individuals were selected on random basis.

Questionnaire based on 15 closed ended questions were asked form the general public covering awareness & history of migraine. several other questions included in the questionnaire such as gender, age group, duration of sickness, occurrence, symptoms, emotional and some of physical factors, inheritance factor and duration of treatment pattern.

**Results**

Results of the current study showed in table 1 that number of female prevalence in our area are more than male where noticed 138 patients (60%) while 92 males registered (40%). in the same table we noticed that women are taking their medicine faster than male. significant different has noticed among females who taken their medicines no more that 2 hours. even with other time point studied in the current study women were taking their medicines. describing pain feeling illustrated that 41% of female pronounce their pain by ache and pressure while 31% explained their pain by throbbing or pounding comparing to male where 29% of them experience their pain as throbbing or pounding. one of survey questions was that is migraine headaches bad to degree of awaken patients in the night. 42% of female answer were never while 63% of them were occasionally awake in the night. on the other hand, males respond showed that 41% were never awake in the night while 39% of them were occasionally awake from pain. knowing patient history would give a clue for prevalence such sickness among genders. having family member having this sort of sickness also included to have answer illustrated in table 1 where 59% of females answer were no and 41% yes. Table 1 also demonstrated that patients take part of this study respond to question asked about if headache cause health disorder that more males and females do not have any effect while 14% of females and males had health disorders.
Table 1: Demograph for participation who having migraine headache

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>(valus)</td>
</tr>
<tr>
<td>Total sample</td>
<td>138</td>
<td>60%</td>
<td>92</td>
<td>40%</td>
<td>0.003</td>
</tr>
<tr>
<td>Period of headaches before take medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No more than 2 hours</td>
<td>34</td>
<td>25%</td>
<td>16</td>
<td>17%</td>
<td>0.045</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>25</td>
<td>18%</td>
<td>17</td>
<td>18%</td>
<td>0.063</td>
</tr>
<tr>
<td>5-12 hours</td>
<td>18</td>
<td>13%</td>
<td>21</td>
<td>23%</td>
<td>0.051</td>
</tr>
<tr>
<td>12-24 hours</td>
<td>32</td>
<td>23%</td>
<td>28</td>
<td>30%</td>
<td>0.067</td>
</tr>
<tr>
<td>Several days</td>
<td>25</td>
<td>18%</td>
<td>9</td>
<td>10%</td>
<td>0.003</td>
</tr>
<tr>
<td>1 week or longer</td>
<td>4</td>
<td>3%</td>
<td>1</td>
<td>1%</td>
<td>0.35</td>
</tr>
<tr>
<td>Describe Migraine Headaches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throbbing/pounding</td>
<td>43</td>
<td>31%</td>
<td>27</td>
<td>29%</td>
<td>0.038</td>
</tr>
<tr>
<td>Ache/pressure</td>
<td>56</td>
<td>41%</td>
<td>39</td>
<td>42%</td>
<td>0.043</td>
</tr>
<tr>
<td>Like a tight band</td>
<td>14</td>
<td>10%</td>
<td>21</td>
<td>23%</td>
<td>0.001</td>
</tr>
<tr>
<td>Dull</td>
<td>20</td>
<td>14%</td>
<td>2</td>
<td>2%</td>
<td>0.01</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>4%</td>
<td>3</td>
<td>3%</td>
<td>0.98</td>
</tr>
<tr>
<td>Is Migraine headaches awoken at night</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>58</td>
<td>42%</td>
<td>38</td>
<td>41%</td>
<td>0.021</td>
</tr>
<tr>
<td>Occasionally</td>
<td>63</td>
<td>46%</td>
<td>36</td>
<td>39%</td>
<td>0.004</td>
</tr>
<tr>
<td>Often</td>
<td>17</td>
<td>12%</td>
<td>18</td>
<td>20%</td>
<td>0.55</td>
</tr>
<tr>
<td>Does family members have migraine headaches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>82</td>
<td>59%</td>
<td>56</td>
<td>61%</td>
<td>0.072</td>
</tr>
<tr>
<td>Yes</td>
<td>56</td>
<td>41%</td>
<td>36</td>
<td>39%</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table2: Times of migraine experienced per month frequent of migraine headache per month would be explain who experienced more impact outcome from this sickness. Our results from table 2 showed that males and females having twice headaches per month is the most common answers 38% followed by once per month in females 18%. 14% of females and 13% of males had migraine headaches 3 times. 12% of females had migraine headaches 4 times per month while 8% of males showed same rate of headaches incidence.

In the present study authors pay intentions to general health of participants. Table 3 showed that period of having migraine headaches for more that 2 hours were higher among females 25% comparing to 17% among males. 18% of males and females had pain for 3-4 hours. 23% of males and females had pain for 5-12 hours of migraine headaches. 18% of females and 10% of males having pain for several days.

Considering general health, thus one of survey questions was that if patients had a head or neck injury.
77% of females and 80% of males did not have any injury while 23% of females and 20% of males had injury in neck and head. Having hormone or vitamins level checked answer was high no answer among females 87% comparing to 65% among males. 13% only of females had positive answer while 32% of males answered with yes. The survey included also question about general health in the last month 49% of females answered with good and 64% of males have same answered. 17% of females and 10% of male answered with excellent. Question about if migraine headaches effect on life quality, the answers were 57% of females it is effect at moderated level while 44% of males were answered at same level. Moderated effect was the higher percentage of impacts followed by 20% of females who answered with extremely effected which less than males at 21%.

Table 3: General health of patients participate in the study.

<table>
<thead>
<tr>
<th>Times</th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>18%</td>
<td>15</td>
<td>17%</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>38%</td>
<td>35</td>
<td>38%</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>14%</td>
<td>12</td>
<td>13%</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>12%</td>
<td>7</td>
<td>8%</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>7%</td>
<td>8</td>
<td>9%</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>4%</td>
<td>4</td>
<td>4%</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>3</td>
<td>3%</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1%</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>4%</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>1%</td>
<td>1</td>
<td>1%</td>
</tr>
</tbody>
</table>

Discussion

Migraine headaches are commonly occurring between the age of 20 to 50 years and this is similar to what has been found in the current study (12). More females that males who suffer from migraine headaches registered in the current study and this come along with previous studies which approved that migraine headaches are affected more on boys than girls before youth but it would increase among women for 2 -3 times more in elderly more than men. It has been reported that around one in each five women practices migraine headache. (13, 14). one study concluded that women are more express migraine attacks than men(13) . The frequencies of migraine headache results showed that significant increase in women in duration of more than 2 hours and several days and there are not different in the different duration included in the current study between males and females. Epidemiological studies in Korea on migraine headache demonstrated that women having long time expressing of headache than men and women also have an around three times greater risk of having migraine (15). Symptoms and description of sort of pain results headaches showed that more women explained their pain by Throbbing or pounding. As well as more women clarified their headache by Ache, pressure, Like a tight band and Dull comparing to males who less complain about headache and they always descripted as head pain. Our finding are in agreement with previous reported females to males description of pain where these studies illustrated that women predominant in groups of individuals that are affected by migraine (16)
up by night because the headache comparing to males. This may be related to male’s work and sustainability to pain. No significant different among females and males who having family history of migraine headache. Previous study showed that there are correlation to migraine headache and family history where they found 4 out of every 10 case of migraine showed headache relative but this study do not showed the different among males and females in this percentile(17). More females do not have health problem than males. Nevertheless, headache frequency and intensity found to be more related with health problems and gender has been approved in previous studies conducted in united states and Germany(18) in the current study, it has been proven that significant increase expressed 2 and 3 times of migraine headaches per month among women comparing to men. Sex specific may different according to hormone different. It has been reported that high level of estrogen and estrogen drawing through the menstrual period would be proposed as an elucidation for increase expression times per month among women (19).

Another question included in the questioner about who long the pain will be stay after taken pain medication, more women than men answered with continuing fell with pain after more than 2 and 3 hours after take medicine and even more women who feel with pain even after several days from taken medicine. This change in responded may be according to differences in the brain structural and neural circuity between women and men(20). Small proportion of patients who suffer from migraine visit doctors and use prescribed medication for migraine(14). No different has been showed among men and women who having injury in neck and head less women than men who do not checked their hormones and vitamins among our participant and this is because regular physical health checked and lab analysis is not common in Iraq, most patients going to doctor when they fell serious pain.

In summery migraine effect more in females comparing to males. Furthermore, we noticed through our analysis that more sex differences associated with some of characteristics and health disorders and family history. Our finding would propose the females are more prominent to headache and we recommended more study in this sickness and more awareness campaigns among Iraq population.

Acknowledgment: I want to express my thanks to the following personnel who participated in this research. Zynab Nadem Baqer, Duaa Jawad Mashkur, and Mohamad Shaker Saleem.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References


Orofacial Tuberculosis: An Uncommon Manifestation of A Common Disease- A Narrative Review

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Abstract
Tuberculosis is chronic multisystemic disease caused mostly by Mycobacterium tuberculosis. The disease has its mention in the Vedas and Chakra and Sushruta described the features in the early 600 B.C. TB has a known site predilection for lungs, although, extra pulmonary cases have also been reported. The oral tuberculous lesions are a rare occurrence with atypical signs and symptoms, thus deferring an accurate diagnosis and treatment. A plethora of standard and contemporary diagnostic adjuncts facilitate the rapid and precise diagnosis. Anti-tubercular regimen forms the mainstay of treatment, although several recent drugs are also in clinical trial phase.

This article aims to highlight the etio-pathogenesis, clinical and oral features of tuberculosis, diagnostic tools, and management protocol for tuberculosis. There is also a brief mention on the various policies considered by World health Organization (WHO) to combat this dreaded disorder.

Key Words: diagnostic tools, oral tuberculosis, pulmonary tuberculosis, treatment protocol, tuberculosis.

Introduction
Tuberculosis (TB), a chronic, multisystemic infectious disease is characterized by granuloma formation.1 Tuberculosis (TB) is considered as a global public health hazard, outnumbering AIDS as the major infectious mortality cause. Early 80’s witnessed the decreased TB incidence due to BCG vaccination, anti-TB therapy and improved health care facilities. Resurgence of TB post 1985 occurred due to increased global population, HIV epidemics, deprived sanitary and health care conditions, TB endemic countries immigrants, and multi drug-resistance to anti TB.2,3 Currently, there is a decline in the incidence of TB cases, although, HIV infections and drug resistant strains pose a threat to the absolute abolition of TB.4

Robert Koch (1882) discovered the etiologic agent for TB.5 National Tuberculosis Association (American Lung Association) was established in 1904.6 Albert Calmette and Camille Guerin (1908) made the historic invention of TB vaccine BCG (Bacillus Calmette Guerin). The vaccine was first used on the human population in 1921. The vaccine is now counted in WHO’s list of most essential medication for basic health system.7

Epidemiology of Tuberculosis
According to the WHO TB Report (2018), TB has affected approximately 10.0 million (range, 9.0–11.1 million) individuals.8 The global tuberculosis burden reveals a marked variation among the numerous nations,
ranging from less than five to more than 500 new cases/100,000 population/year (130 being the average global burden). In 2018, TB associated mortality accounted for 1.2 million (range, 1.1–1.3 million) deaths among HIV-negative individuals (a 27% reduction from 1.7 million in 2000), and 2,51,000 deaths (range 2,23,000–2,81,000) among HIV-positive cases (a 60% reduction from 620,000 in 2000) respectively.9 Both sexes in all age groups are affected by TB, Men (aged ≥15 years) followed by women and children (aged <15 years) attributed for 57%, 32%, and 11% of all TB cases respectively. Among all TB cases, 8.6% patients had associated HIV infection (PLHIV).

Most TB cases in 2018 belonged to South-East Asia (44%), Africa (24%) and the Western Pacific (18%), with smaller percentages in the Eastern Mediterranean (8%), the Americas (3%) and Europe (3%). Eight countries accounted for two thirds of the global total: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%).10

India accounted for 35% of global TB deaths among HIV-negative people, and for 30% of the combined total number of TB deaths in HIV-negative and HIV-positive people. In India, notifications increased from 1.2 million in 2013 to 2 million in 2018 (+60%), including a 12% increase of 2,07,000 between 2017 and 2018. (https://www.who.int/tb/publications/global_report/en/)

Uttar Pradesh, with 17% of population of the country, is the largest contributor to the TB cases with 20% of the total notifications, accounting to about 4.2 Lakh cases (187 cases/lakh population). (https://tbcindia.gov.in/WriteReadData/India%20TB%20Report%202019.pdf)

Etiopathogenesis

The primary causative agent for tuberculosis is Mycobacterium tuberculosis.11 Mostly, transmission occurs via the respiratory route due to inhalation of Mycobacterium tuberculosis infected air droplets.12 Intake of unpasteurized cow’s milk (infected by Mycobacterium bovis) or infection by other atypical mycobacteria may also transmit the disease.13

Inhalation of contaminated air droplets causes seeding of M. tuberculosis bacilli in the lung alveoli. In immunocompetent individuals, the body defense mechanism (alveolar macrophages) clear this infection. However, in immunocompromised patients, M. tuberculosis multiplies inside the macrophages, and gets disseminated to regional lymph nodes, with further infection spread to the lungs, vertebrae, peritoneum, meninges, liver, spleen, and genitourinary tract. Cell-mediated immunity develops at this time and diagnostic tests of tuberculosis become positive. Tuberculosis pathogenesis usually ceases at this stage. The individual is symptom free and is said to have tuberculosis infection.14

In few cases, tuberculosis infection shows further progression to tuberculosis disease. If a healthy adult acquires M. tuberculosis infection, there is 5% to 10% likelihood that TB disease may develop during their life. There is 40% to 50% probability that TB disease may develop within 6 to 9 months in infected but untreated infants and toddlers.15 Several conditions may predispose to immune suppression (HIV infection, diabetes mellitus, malnutrition), causing progression from infective phase to disease in adults and children.16

TB pathogenesis is depicted in Fig.1.
TYPES OF TUBERCULOSIS- PULMONARY AND EXTRA PULMONARY TUBERCULOSIS

TB can be classified as either pulmonary or extra-pulmonary, based on the primary organ system implicated. Pulmonary TB is the most frequently encountered form. Tuberculosis may affect several extrapulmonary sites such as Lymph nodes, peritoneal cavity, genitourinary, nervous system, musculoskeletal system, and hepatosplenic systems.  

Approximately about 25% of TB lesions are seen in extra pulmonary sites, and head and neck tuberculosis occur in 10- 15% cases. However, primary oral lesions account to less than 1% of primary head and neck lesions. Oral tuberculous lesions may occur either primary or secondary to pulmonary tuberculosis with secondary lesions being more common. Primary oral tuberculous lesions have an age and site predilection (seen in younger age group, and gingiva being the commonest site), with accompanying regional lymphadenopathy. Tongue is the primary involved site in secondary TB, although palate, lips, buccal mucosa, gingiva and frenum may also be affected.

Oral tuberculous lesions usually have diverse manifestations, causing diagnostic dilemmas particularly when oral lesions herald the systemic features. Hence, TB should be considered in the differential diagnosis of suspicious oral pathologies.

Orofacial TB may involve any structure in and around the oral cavity. Andrade et al. suggested a classification of orofacial TB based on the site involved.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Lumpy jaw, presenting as an extra oral swelling (No draining sinus tracts intraorally/extra orally).</td>
</tr>
</tbody>
</table>
| II    | History of extraction  
Non-healing extraction sockets with/without single/multiple draining intraoral/extraoral sinus tracts |
| III   | No history of extraction  
Intraoral / extraoral draining sinus / sinuses in the orofacial region and an osteomyelitis bony lesion |
| IV    | Tuberculous lymphadenitis of the orofacial region  
No manifestations of Type I, II or III |
| V     | TB lesions in and around the oral cavity. |

Oro-facial TB manifests in various forms: Tuberculous ulcer, Desquamative gingivitis and gingival enlargement, lymph node TB, tuberculosis of tooth socket, tuberculous osteomyelitis, tuberculous sialadenitis, tuberculosis of maxillary sinus, tuberculous involvement of the Temporomandibular jaw and tuberculosis of skin.

1. **Tuberculous Ulcer**

Tuberculous ulcer is the most common oral lesion, appearing as an opalescent vesicle/nodule in its prodromal phase, which eventually rupture forming an ulcer. Tuberculous ulcer typically manifest as an irregular ulcer with undermined edges, mild indurated base covered with a yellowish granular slough.

Primary tubercular ulcers are rare, seen primarily in children and young individuals, appearing as shallow/deep, non-tender ulcers with tender lymph nodes. However, secondary lesions are seen in elderly as painful
ulcers with undermined margins, indurated base, covered with yellowish slough, and non-tender lymph nodes.23

The most common site for tuberculous ulcers is the tongue (lateral border ≤ tip ≤ anterior dorsum ≤ ventral surface), where it occur as persistent non-healing ulcer.24 Tuberculosis of the tongue may also manifest as an enlarged tongue (Macroglossia).19 Traumatic ulcers, aphthous ulcers, syphilitic ulcers and malignant ulcers should be considered in the differential diagnosis of TB ulcers.13

2. Tuberculous Gingivitis

Gingival tuberculosis may present as solitary proliferating granulation tissues or as mucosal ulcerations/erosions with rare concurrent marginal periodontitis.25,26 Desquamative gingivitis may occur in chronic infections, with tuberculosis being the most common chronic infection. Hasan et al. reported an interesting case of oral TB presenting as desquamative gingivitis.19 There are documented cases of gingival tuberculosis appearing as diffuse gingival enlargement.27,28

Gingival tuberculosis is rare and should be considered in the differential diagnosis of a persistent, recalcitrant, non-healing lesion.29,30

3. Tuberculoma

Tuberculous bacteria may reach the periapical tissues of the jawbone via-

A) Salivary acid-fast bacilli may invade the pulp of a deeply decayed tooth causing a periapical tuberculous periapical infection.

B) Blood stream TB dissemination

C) Deep periodontal pocket.31

The lesions are painless, rapidly spreading with significant bone involvement.19

4. Tuberculous involvement of extraction sockets of teeth

Deferred healing causes replacement of the extraction socket with pinkish-redish “tuberculous granulation tissue”.32 TB in such cases may result from the treating dentist who was later diagnosed with active pulmonary TB.33

5. TB lymphadenitis

Lymph node tuberculosis (LNT) is the most frequently occurring form of extra pulmonary TB in low TB prevalence regions. In TB endemic countries, LNT is outnumbered only by TB pleuritis. Mycobacterial cervical lymphadenitis is the most common form of LNT.34 TB lymphadenitis is atypical in its affinity to sex and age, primarily affecting younger age group
females, whereas pulmonary tuberculosis affects older male individuals.35

LNT presents as gradually progressing painless swelling of one/more lymph nodes. Initially, presents as firm, distinct, and mobile LN, later, appearing as matted with inflamed overlying skin. The full-blown cases manifest as softened lymph nodes with abscess and sinus.1

Jones and Campbell classification36 for LNT -

- Stage 1 - Reactive lymphadenitis - distinct, enlarged, mobile and firm nodes with nonspecific reactive hyperplasia
- Stage 2 - Peri adenitis - Rubberly lymph nodes fixed to underlying tissues
- Stage 3 - Cold abscess formation with softened central region
- Stage 4 - collar-stud abscess
- Stage 5 - Sinus tract formation.

The varied treatment protocol of tuberculous and nontuberculous mycobacterium cervical lymphadenitis warrants the differentiation between the two entities.37

6. TB SALIVARY GLANDS

Salivary gland TB is rare even in TB rampant nations like India. (2.5-10%).38 Primary and systemic TB has a site predilection for Parotid and Submandibular glands, respectively.39,40

According to Van Stubenrauch theory, the infection spreads from the oropharynx along the parotid gland duct (Stenson’s duct). According to Bockhorn and Berman postulates, dissemination of infection is through the blood stream and lymphatics, respectively.41

Tuberculous parotitis is frequently due to Mycobacterium bovis infection and rarely by Atypical mycobacterium.42 Parotid TB manifests in the following forms-

1. Acute inflammatory reaction of the gland mimicking sialadenitis, where the gland parenchyma forms multiple small abscess, later resulting in diffuse gland enlargement.43

2. Well circumscribed mass presenting as a progressively enlarging, asymptomatic mass, mimicking parotid neoplasm.44

3. Rarely as fistulous tract / abscess in the periauricular region.45

7. TUBERCULOUS OSTEOMELITIS

Local and systemic factors (viral infections, blood dyscrasias, chemotherapy and radiotherapy) predisposing to immune suppression usually aid in development of acute and secondary chronic osteomyelitis of the jawbones.46

Tuberculous jawbone osteomyelitis accounts only to less than 2% of skeletal tuberculosis.47 Maxillary jawbone TB osteomyelitis is rare mainly due to rich vascularity and strut maxillary bone structure.48

TB jawbone osteomyelitis typically present as apical osteitis and periodontitis with horizontal bone loss or as an extensive destructive osteolytic lesion, and differentiation with a dental abscess is difficult.49

8. TUBERCULOSIS OF TEMPOROMANDIBULAR JAW

TMJ tuberculosis usually occur secondary to a fistulous communication from the middle ear,50 and only six cases have been described as primary TB of the TMJ.51 A recalcitrant, painful preauricular swelling associated with trismus is the most frequently occurring manifestation.52 TB of the TMJ must be considered in the differential diagnosis of acute TMJ swelling and bone destruction, especially in TB endemic regions or those at risk of HIV.53

Initially, nocturnal muscle spasm occurs causing soft and elastic joint destruction, eventually causing localized atrophy of the periarticular muscles.54 Fibrosis or bony ankylosis, causing erosion of the condyle and glenoid fossa occurs in the advanced cases.

9. LUPUS VULGARIS

Oral lupus vulgaris is an exceptionally rare, however, it is the most common type of cutaneous TB in people with moderate immunity and high tuberculin sensitivity.57 In India, the common affected sites are buttocks, thighs, and legs, with rare facial involvement.
Lip mucosa, palate and the gingiva are the common oral sites affected. Disseminated pre-existing skin lupus outbreak, bacterial seeding from contaminated sputum, and hematogenous spread from the primary site may be the proposed pathogenic mechanisms. Laskaris described 4 cases of lupus vulgaris of the oral cavity.

Lupus vulgaris characteristically presents with a reddish-brown, nodular/plaque-like lesion, and shows an “apple-jelly” color on diascopy test.

10. TB NOSE AND PARANASAL SINUSES

Nasal and paranasal sinuses TB occurs infrequently due to the defensive mechanisms of the nasal mucosal ciliary action (nasal vibrissae) and the nasal secretions bactericidal properties. Maxillary and ethmoid sinuses are most frequently involved.

Maxillary sinus TB generally occur secondary to pulmonary tuberculosis. Nasal blockage/discharge/bleeding (epistaxis) with crusting are the common presenting manifestations. Sinonasal TB occur in 3 major forms: (i) mucosal involvement with polyp formation (ii) bony involvement with midfacial defects and fistular tracts (iii) hyperplastic form with granuloma impersonating a malignant lesion.

DIAGNOSTIC AIDS FOR OROFACIAL TUBERCULOSIS

TB diagnosis is usually made by detailed medical history, Physical examination and diagnostic aids like microscopic sputum smear examination, chest radiography and culture methods.

Fig. 3 depicts the various diagnostic aids in orofacial tuberculosis.
Fig. 3 Diagnostic Aids of Orofacial TB

**Prevention of Tuberculosis**

Center for disease control and prevention (CDC) has issued certain precautionary measures for healthcare professionals. Elective procedures should be deferred for active/suspected TB cases, and until all the TB investigations are negative. However, mandatory emergency treatment may be performed with certain precautionary measures:

- **a)** Appointment at the end of the day
- **b)** Should be the last treated patient
- **c)** Universal precautions for infection control-
  - Sterilization and disinfection of the operation theatres and instruments.
  - **Use of Rubber dams to prevent aerosol contact (except in patients with evident coughing)**
  - **Proper hand hygiene care**
  - **personal protective equipment (eye shields, HEPA or NIOSH N 95 face masks, head caps, gloves, and surgical gowns)**
  - **Evade direct contact with blood, body fluids and mucous membranes.**

**BCG VACCINATION**

Mycobacterium bovis Bacillus Calmette Guerin (BCG) vaccine is the most common preventive method.
to control global tuberculosis. BCG prevents severe disease and reduces death rates from miliary TB among children and meningeal TB in newborns, with variable suboptimal protection against pulmonary tuberculosis in children or adults. The Modified-Vaccinia-Ankara (MVA) 85A vaccine is an attempt to develop superior novel vaccine. However, clinical trials and studies have shown that it does not provide enough protection against TB.

### TREATMENT PROTOCOL

Anti-tubercular therapy (ATT) regimen varies based on the stage of infection and the individual’s risk likelihood. ATT regimen usually entails a drug combination, or a mixture of several drugs in a phasic manner (initial 2-month intensive phase followed by a 4- to 6-month continuation phase).

Directly observed therapy short-term (DOTS) efficiently monitors treatment adherence and completion. With the emergence of MDR-TB, DOTS-plus is a more arduous treatment strategy.

### Table 2 Management of orofacial tuberculosis

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>MECHANISM OF ACTION</th>
<th>ADVERSE DRUG EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FIRST LINE DRUGS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Ethambutol</td>
<td>Inhibits arabinosyl transferase</td>
<td>Optic neuritis, loss of visual acuity</td>
</tr>
<tr>
<td>2. Pyrazinamide</td>
<td>Inhibits fatty acid synthetase</td>
<td>Morbilliform rash, Arthralgias, Hyperuricemia</td>
</tr>
<tr>
<td>3. Isoniazid</td>
<td>Inhibits fatty acid synthetase</td>
<td>Hepatitis, Peripheral neuropathy Inhibits cytochrome P450 enzymes</td>
</tr>
<tr>
<td>4. Rifamycins: Rifampin, Rifabutin, Rifapentin</td>
<td>Binds to RNA Polymerase and inhibits transcription</td>
<td>Hepatitis, Flu-like symptoms Reddish urination, GIT disturbances</td>
</tr>
<tr>
<td><strong>SECOND LINE DRUGS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Cycloserine</td>
<td>Inhibits monomer synthesis</td>
<td>Psychosis, Seizures, Peripheral neuropathy</td>
</tr>
<tr>
<td>2. Ethionamide</td>
<td>Inhibits fatty acid synthetase</td>
<td>Hepatitis, Hypothyroidism</td>
</tr>
<tr>
<td>3. Aminoglycosides: Streptomycin, Capreomycin, Kanamycin, Amikacin</td>
<td>Binds to 30s ribosomal units and inhibit translation</td>
<td>Ototoxicity, Nephrotoxicity, Neuromuscular blockade</td>
</tr>
</tbody>
</table>
4. Fluoroquinolones:  
Ciprofloxacin  
Oﬂoxacin  
Gatifloxacin  
Levoﬂoxacin  
Moxiﬂoxacin  

- Inhibits topo-isomerase II (DNA Gyrase), thereby releasing DNA with staggered double stranded breaks  
- Nausea, Abdominal rashes, Restlessness, Confusion

**COMBINATION DRUGS:**  
Rifamate  
Rifater  

- Competitive para-amino benzoic acid antagonist  
  - Isoniazid+Rifampin  
  - Isoniazid+Rifampin+pyrazinamide  
- GIT disturbances

Persistence and resistance are the primary factors preventing complete TB eradication. The bacilli evoke a chronic inflammatory reaction, causing the bacillary sequestration and preventing drug exposure. This requires extended ATT therapy to eliminate the bacterium, avoiding recurrence.74

Drug resistance occurs due to gene mutations, causing a heritable loss of drug susceptibility. Two types of drug resistance are observed in the context of TB.

MDR-TB- Mycobacterium tuberculosis (M. tuberculosis) is resistant to the most efficient first-line ATT.

XDR-TB has additional multi-drug resistance to the most active second-line agents, and fluoroquinolones.75

Researches are being conducted to develop novel anti-TB drugs that are economic, have better efficacy, a shorter treatment course, and should be able to treat latent TB, MDR-TB, and XDR-TB.76

**Table 3 Tabular representation of the recent TB therapeutic development.**

<table>
<thead>
<tr>
<th>Drug category</th>
<th>Mode of action</th>
<th>Brand name</th>
<th>Phase of clinical trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaryquinoline</td>
<td>Inhibits ATP synthesis with disrupted membrane potential</td>
<td>Bedaquiline</td>
<td>III</td>
</tr>
<tr>
<td>Nitroimidazoles</td>
<td>Mycolic acid synthesis inhibition. Metabolization releases nitric acid resulting in bacterial cell wall poisoning.</td>
<td>Delamanid PA-824, OPC-67683, TBA 354</td>
<td>III II, II, Preclinical</td>
</tr>
<tr>
<td>Fluroquinolones</td>
<td>Inhibits DNA synthesis</td>
<td>Gatifloxacin, moxifloxacin</td>
<td>III</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>Inhibits cell wall synthesis</td>
<td>SQ 109</td>
<td>II</td>
</tr>
<tr>
<td>Rifamycin</td>
<td>Blocks transcription by inhibitory action on bacterial DNA dependent RNA polymerase.</td>
<td>Rifapentin</td>
<td>II/III</td>
</tr>
</tbody>
</table>
FUTURE PERSPECTIVES AND GOALS TO COMBAT TUBERCULOSIS

World Health Organization (WHO) has adopted Global strategy and targets for tuberculosis prevention, care, and control in 2015 (The End TB Strategy).

https://www.who.int/tb/strategy/End_TB_Strategy.pdf?ua=1

The framework of the post-2015 global tuberculosis strategy is presented in Table 4

<table>
<thead>
<tr>
<th>Vision</th>
<th>Tuberculosis free world</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Zero mortality, ailment, and affliction due to tuberculosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Goal</th>
<th>End the global tuberculosis epidemic</th>
</tr>
</thead>
</table>

| Milestone for 2025 | 75% reduction in tuberculosis deaths |
|                   | 50% reduction in tuberculosis incidence rates (less than 55 TB cases/100,000 population). TB affected families not to face calamitous charges. |

| Targets for 2035 | 90% reduction in tuberculosis deaths |
|                 | 90% reduction in tuberculosis incidence rates (less than 10 TB cases/1,00,000 population) TB affected families not to face calamitous charges. |

Conclusion

Tuberculosis is a common multisystemic disease, primarily affecting the pulmonary system. Oral TB lesions have bizarre clinical presentation, and the dentists need to be well acquainted with the varied oral features to arrive at an early and accurate diagnosis and treatment. Drug resistance to the various anti-tubercular drugs has posed a serious concern, and several novel ATT are currently in clinical trial phases to combat this complication. WHO has also adopted Global strategy and targets for tuberculosis prevention, care, and control.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Nil

Ethical Clearance: Not Applicable as it is a narrative review.

References


Role of Vitamin D For Oral Health and Overall Health

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Abstract

Vitamin D, sometimes called the “sunshine vitamin” is produced in our skin in response to sunlight. It’s a fat-soluble vitamin in a family of compounds that includes vitamins D-1, D-2, and D-3. Our body produces vitamin D naturally when it’s directly exposed to sunlight. Vitamin D has several important functions. Perhaps the most vital are regulating the absorption of calcium and phosphorus, and facilitating normal immune system function. Getting a sufficient amount of vitamin D is important for normal growth and development of bones and teeth, as well as improved resistance against certain diseases. Nowadays, Vitamin D deficiency patients are identified in various medical fields and it is necessary to understand the symptoms, pathophysiology and therapeutic measures to overcome the deficiency of this vitamin in various age groups from pediatric to geriatric.

Key words: Cholecalciferol, Deficiency, Geriatric, Pediatric

Introduction

Vitamin D which was once known as “Fat soluble vitamin” now has been reincarnated with a dual role as “hormone” as well. Low vitamin D levels can drastically impact a person’s physical and mental well-being. Recently, following the discovery of vitamin D receptors throughout the body, its role in the prevention and treatment of chronic diseases has become an important area of research and interest. Vitamin D deficiency has been associated with various health problems such as cognitive decline, depression, osteoporosis, cardiovascular disease, hypertension, diabetes, and cancer. The process of aging predisposes the risk for vitamin D deficiency. Vitamin D plays a role in maintaining the homeostasis of various biological systems including the neuromuscular, skeletal, cutaneous, cardiovascular, and immune systems. Vitamin D also has properties such as tumor suppressing, anti-inflammatory, and antibacterial properties. It also plays an important role in Dentistry, in the development of teeth, promotion of the immune response to oral microbial infections, and promotion of healing post oral surgery. This article deals with the etiology, pathophysiology of vitamin D deficiency and its role in oral health from pediatric to geriatric.

Discussion

Vitamin D in its inactive form (vitamin D₃ or Cholecalciferol) is a steroid hormone that is synthesized in the skin with adequate exposure to the sun (ultraviolet light-bandwidth or frequency needs mentioning) and/or acquired through diet1-5. Foods naturally containing vitamin D are rare, and it can be found in high quantities in oily fish (such as salmon, mackerel, and herring) and commercial oils from fish (e.g., cod liver oil)⁶. Some of the major causes of VDD (Vitamin D Deficiency) is the lack of exposure to sunlight with adequate ultraviolet B rays (exogenous factor). VDD can also arise from a nutritional deficit due to inadequate intake of vitamin D, or hereditary disorders due to intestinal malabsorption and metabolic disorders. Drug related VDD is also possible due to iatrogenic causes such as increased clearance with anti-convulsant drugs phenytoin, carbamazepine, oxcarbazepine.

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DOI Number: 10.37506/ijfmt.v15i1.13672
Vitamin D comprises of two components namely Vitamin D2 and D3. Vitamin D2 is produced via ultraviolet irradiation of ergosterol from yeast, while Vitamin D3 is obtained from ultraviolet irradiation of 7-dehydrocholesterol from lanolin, exhibiting the biological activity of cholecalciferol (vitamin D3), and it is synthesized in the human skin. According to the Endocrine Society Clinical Practice Guidelines, Vitamin D deficiency is defined as levels of 25(OH)D below 50 nmol/L and insufficiency as 25(OH)D levels of 52.5–72.5 nmol/L. The biomarker used for analysis of vitamin D status is by measurement of serum 25-hydroxyvitamin D (25[OH]D).

The cellular actions of Vitamin D are mediated through the Vitamin D receptor (VDR), which is a receptor molecule that binds to the active form of Vitamin D through the Vitamin D receptor (VDR), which is a ligand (RANKL). Mature osteoclasts remove calcium and phosphorus from the bone, maintaining the serum levels of calcium and phosphorus by hydroxylase (24-OHase), upholding its excretion in the bile. 1,25(OH)2D is recognized in the osteoblasts, 25-hydroxyvitamin D-25-hydroxylase (VD-25-hydroxylase) to vitamin D–binding protein (VDBP), which transports it to the liver. There, Vitamin D is converted by vitamin D-25-hydroxylase (VD-25-hydroxylase) to 25- hydroxyvitamin D (25(OH)D) (used as standard marker to the fetus vitamin D follow the maternal concentration and can be used as a standard marker to the fetus vitamin D deficiency will need to be considered with special focus on vulnerable population such as children, pregnancy, immuno-compromised individuals. With respect to oral disease, caries and periodontal disease are associated with VDD and its pathophysologic processes. Teeth are mineralized organs, surrounded by alveolar bone, and formed by three distinctive hard tissues: enamel, dentin, and cementum. The tooth mineralization process occurs parallel to skeletal mineralization, yet if mineral metabolism is disturbed then failures will occur similarly to those that occur in bone tissue. Vitamin D has impact on oral health based on bone metabolism. VDD compromises odontogenesis, resulting in a hypo mineralized dentition susceptible to fracture and caries lesions. Vitamin D plays a key role in bone and tooth mineralization, and when levels are unregulated it can lead to the “rachitic tooth”, which is a defective and hypo mineralized organ highly susceptible to fracture and decay.

Deciduous dentition can be influenced by maternal 25(OH)D levels, despite the influence of inherited defects of the fetus. Fetal serum-circulating levels of vitamin D follow the maternal concentration and can be used as a standard marker to the fetus. Therefore, if maternal 25(OH)D levels become unbalanced, this may have direct effect on the baby’s health particularly on tooth development. Nowadays, it is known that maternal VDD at 12–16, 20–32 and 36–40 weeks results in defects at the incisal third, middle third and cervical third of crowns respectively.

Vitamin D deficiency can impair the immune response to oral microbial infections, increasing the risk of oral infections and periodontitis. According to WHO, Dental caries is the fourth-most expensive chronic disease to treat. It has a complex and multifactorial etiology. Factors, such as cariogenic diet with a high carbohydrate content, cariogenic bacteria, and poor oral hygiene are risk factors for caries and periodontal infection. Certain evidence also highlights the association of low levels of vitamin D and the high prevalence of caries in both children and adults, although the mechanism remains unclear.

Hujoel et al concluded from studies that optimal vitamin D concentration (≥75 nmol/L) is associated with lower odds for dental caries in children. A randomized controlled trial concluded that the vitamin D supplementation reduced the risk of caries in about 47%, but with low certainty. Schroth et al showed that caries-free children were twice as likely to have optimal vitamin D concentrations (≥75 nmol/L) and those with severe early childhood caries were at nearly three times the odds of having deficient levels (<35nmol/L).
The impact of nutrition on periodontal health, particularly Vitamin D deficiency, has been investigated and a recent European study stated that an inadequate vitamin D status impacts periodontal health and oral functions. Vitamin D concentrations were associated with higher periodontal destruction, severe periodontitis stages and higher tooth loss. A more recent study from 2019 showed vitamin D supplementation was linked to a decrease of salivary cytokines before nonsurgical periodontal treatment. The association between periodontitis and maternal VDD reveal that pregnant women with moderate to severe periodontitis are diagnosed with lower serum levels of vitamin D. Non-surgical periodontal treatment during pregnancy was proved to be successful in reducing adverse pregnancy outcomes along with vitamin D supplementation showed mild clinical improvements in birthweight as shown by Khan et al.

Movement of tooth depends on the application of predetermined forces that cause mechanical stimuli with two simultaneous processes such as bone resorption on the pressure site, through osteoclastic activity; and bone formation on the tension site, by osteoblastic action. These processes may result in rapid tooth movement. Albeit animal observational in nature, there is an increasing evidence from a study by Kale et al showing that local application of vitamin D results in a faster tooth movement.

VDD is common in patients with oral neoplastic lesions. In a case-control study, direct association between VDD and increased risk of squamous cell carcinoma of the esophagus, oral, and pharyngeal cancers, which were more prevalent in heavy smokers and severe alcoholism. Anand et al demonstrated that vitamin D receptor expression was increased in premalignant lesions and oral cancer, and vitamin D supplementation significantly diminished therapy-related toxicities in late-stage oral cancers, with less morbidity and better quality of life.

Vitamin D is well known to be essential for the geriatric population. Osteomalacia in adults, like childhood rickets, develops in vitamin D deficiency, commonly presenting with severe aches in bones and muscles, marked proximal muscle weakness making standing up and walking difficult and painful and a marked ‘waddling’ gait. This condition is common in older people, vitamin D repletion is associated with increased risk, and severity of osteoporosis. Better vitamin D availability may contribute to reducing chronic inflammatory problems such as periodontitis and atheromatous disease, and may reduce acute vascular events due to arterial plaque disruption where inflammation is a major factor in progression of atheromatous disease.

Low baseline vitamin D status predicts reduced healing in the year after surgery for periodontitis, a common problem in older people, and giving vitamin D to subjects with severe periodontal problems improved post-operative bone defect resolution, supporting suggestions that hypovitaminosis D may worsen periodontitis. In Dental implants, osseointegration depends on bone metabolism, there is a possibility that low levels of vitamin D in the blood can negatively affect healing processes and new bone formation on the implant surface. The relationship between serum levels of vitamin D and osseointegration of dental implants is controversial and has been evaluated in a few case reports and animal studies. Most studies suggest that adequate serum levels of vitamin D can enhance the healing of peri-implant bone tissue. Patients with vitamin D deficiency (serum levels of vitamin D <10 ng/mL) showed an early implant failure rate of 11.1% Vs. failure rate of 2.9% in patients with normal levels of the vitamin (>30 ng/mL).

Owing to the impact of vitamin D deficiency-related complications and failures in dentistry, it is necessary that the clinician uses vitamin D supplements when deficiency is observed. Typically, 5,000 IU/day is recommended by the AACE (American Association of Clinical Endocrinologists) but a 8 to 12-week supplementation period is needed to reach adequate levels. This timeframe makes implant dentistry quite inconvenient, owing to the often-encountered need to restore teeth at earlier time points, along with the necessity to satisfy patient expectations within reasonable time frames. Over the years, it has become increasingly clear that vitamin D absorption is further optimized with several co-factors. These include vitamin K, magnesium,
calcium, manganese, and boron, among others. These co-factors, when present, help absorb vitamin D toward optimal levels in shorter healing periods.

Interestingly, the striking overlap between risk factors for severe COVID-19 and vitamin D deficiency, including obesity, older age, has led some researchers to hypothesize that vitamin D supplementation could hold promise as a preventive or therapeutic agent for COVID-19. However, a study conducted by Meltzer et al. in September 2020 concluded that persons who are likely to have deficient vitamin D levels at the time of COVID-19 testing were at substantially higher risk of testing positive than those with sufficient levels, and patients under treatment for vitamin D deficiency were not found to have increased risk for COVID-19. Further research is underway to confirm the efficiency of vitamin D supplementation and its role in prevention of COVID-19 infection, which is a very challenging area of research for the researchers in this current pandemic.

**Conclusion**

It’s imperative to understand the importance of vitamin D deficiency, its role in metabolism, growth, in maintaining the milieu interior in a wide range of population from pediatric to geriatric age groups, where particularly vulnerable age groups are now identified. Its role in regulating the immunopathology inflammatory response in various systems of the body also shows its antioxidant property. It is necessary to adhere to the daily recommendation of vitamin D according to the age of the individual for the maintaining of bone health.

This review article aims to sensitize the clinician about the metabolism, the pathway and the current evidence on vitamin D and the panorama of diseases and disorders that it may bring about and its role in oral health with particular emphasis on dental health. Role of vitamin D has gained momentum and it has now become the norm for it to be supplemented and deficiency treated to achieve optimal outcomes both in well-being and interventions.

**Ethical Clearance:** Nil

Not required as it is a review article

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Knowledge, Attitude and Practice Towards Management
of Trauma to Anterior Teeth among Faculty Members and
Undergraduate Students in Dental and Medical Institute in
South India

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Abstract

Introduction: Traumatic dental injuries are common among children owing to their developing
neuromusculoskeletal responses. This study aims at assessment of knowledge, attitude and practice towards
management of trauma to anterior teeth among faculty members and students of Medical College.

Materials and Method: A cross sectional survey with pretested questionnaire was used to assess the
demographics of the participants along with their knowledge regarding dental trauma, their attitude and
practices to handle them. Total of 200 participants comprising, 150 students and 50 faculty members of
the dental institute were part of this study. Descriptive statistics was used to represent each category. Also,
inferential statistical methods were used to assess any significant differences between the groups when
significance level is kept at 5%.

Results: The findings showed that almost 40% of the participants had experienced dental trauma before,
the most common trauma that they encountered was tooth fracture. Faculty members had better knowledge
score compared to students those who have had previous knowledge before regarding this issue also had
better knowledge than their counterpart.

Conclusion: The present study showed that the participants have sufficient knowledge about dental trauma,
and they showed a positive attitude towards it. However, some were still reluctant to perform dental first aid
when required due to the lack of training availability and education. Dental first aid needs to be a part of the
curriculum. Training programs are even more helpful, as it will help to build the confidence of the operator.
This will create awareness among the public about the importance of managing dental trauma of the anterior
teeth.

Key words: Emergency management; Tooth injuries; Tooth fracture; Tooth avulsion; First aid.

Introduction

Traumatic dental injuries are very common; epidemiological studies revealed that one out of two
children sustains a dental injury, most often between
Dental injuries can be due to a fall, injury from a variety of sports, road accidents, or even being struck by an object. Among them, fall is the most common cause of dental injuries. Crown fractures and luxation are the most commonly occurring dental injuries. Anterior teeth are most commonly involved in both the primary and permanent dentition in the majority of trauma cases. The maxillary central and lateral incisors were the most common teeth injured. The prognosis of traumatized teeth depends on the prompt and appropriate treatment, which often depends on the knowledge of the people managing the victim, which often are school teachers and parents. The International Association of Dental Traumatology (IADT) have come up with dental trauma guidelines that are intended to provide information for health care providers caring for patients with dental injuries. Although the actions taken at the site of accident holds crucial position in the outcome. Therefore, dentists should give appropriate advice in addition to increasing public awareness through electronic communications. Hence, the goal of these guidelines is to provide information for the immediate and urgent care of traumatic dental injuries.

The aim of current study was to assess the knowledge, attitude and practices towards management of anterior teeth trauma among students and faculty of medical institute in Karnataka with the objective to assess if any discrepancy between the students and faculty knowledge, attitude and practices.

Materials and Methods

A total of 200 respondents were selected for the study purpose using purposive sampling technique. Prior to the commencement of study, ethical clearance was obtained from institutional ethical committee. The participants were briefed about the study procedure and written consent was obtained to be a part of the study. A questionnaire was used comprising of 8, 4 and 4 questions for knowledge, attitude and practices. This questionnaire was tested for validity and reliability, which was found satisfactory.

For data presentation purpose, collected data was entered into Microsoft Excel 2016 and subjected to descriptive analysis. Furthermore, data was subjected to SPSS software to calculate correlation if any between the students and faculty.

Results

Table 1 shows that 79 participants have experienced or witnessed dental trauma before, while 121 have not. Out of these two groups, the ones who have had previous experience with dental trauma before shows better mean score of knowledge than the other with p-value of (P=0.009). This has been represented in a bar graph shown in Figure 1.

| Table: 1 Mean scores on knowledge of managing Dental First aid according to background data of study participants |
|---------------------------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Category of Study Participants                              | N   | Mean Score, SD# | Mean diff. | t   | df  | sig. (2 tailed) |
| Faculty Students                                             | 50  | 28.02 ± 3.6 26.91 ± 3.2 | 1.113       | 2.05 | 198 | 0.042*         |
| Students                                                     | 150 |                |             |     |     |                |
| Previous Knowledge of First Aid                              | Yes | 28.3 ± 3.3 26.93 ± 3.3 | 1.365       | 2.259 | 198 | 0.025*         |
|                                                     | No  |                |             |     |     |                |
| Experience / Witnessed Dental Trauma                        | Yes | 27.95 ± 3.5 26.6 ± 3.1 | 1.26        | 2.64 | 198 | 0.009*         |
|                                                     | No  |                |             |     |     |                |

*significant at P ≤ 0.05
% - Percentage, N – Frequency

Figure 1: Background data of study participants (N = 200)

Figure 2: Response by study participants on “need for knowledge on Dental First Aid”
Figure 3: Response by study participants on “Need to include Dental First Aid in Curriculum

Figure 4: Response by study participants on “their reluctance to perform dental first Aid.”
Figure 5: Self-rating of knowledge on Dental First Aid among study participants (Percentage)

Figure 6: Reasons for lack of knowledge on dental first aid among study participants
(Those rating average and below, N = 189)
Figure 2 and Figure 3 show positive responses from faculties and students for the need of knowledge on dental first aid and the need to include dental first aid in curriculum.

Figure 4 shows the majority of the faculties and students are not reluctant to perform dental first aid with recorded percentage of 74% from faculties and 55.3% from students. The remaining 26% of faculties and 44.7% of the students are reluctant to do so.

Figure 5 shows a pie chart of participants self-rated their knowledge on dental first aid. The highest percentage of self-rating by the participants is ‘Average’ (44%) followed by ‘Below average’ (28.5%), ‘Poor’ (21.5%), ‘Good’ (5.5%) and ‘Excellent’ (0.5%).

Figure 6 shows a pie chart of reasons for lack of knowledge on dental first aid among study participants. The highest percentage of reason reported by participants is ‘Lack of professional training’ (63.49%) followed by ‘Lack of dental incidence’ (13.75%), ‘Lack of interest’ (12.6%), ‘Busy curriculum’ (8.9%) and ‘Don’t know’ (1.05%).

Discussion

Dental trauma is defined as traumatic injuries to the oral tissues such as the tooth, gums, alveolar bone and others. This type of trauma usually occurs during sports or rigorous activities, and they commonly affect the younger age groups such as children. The cause of dental trauma is unpredictable and can occur anytime, anywhere and to anyone and the most common tooth involved in dental trauma is the upper anterior, which can lead to negative effects to a person such loss of confidence and difficulty in eating. It is important for the public to know more about the management of the traumatic events and preventive measures that can be taken, so that the incidence of dental trauma can be reduced. Hence, this study was carried out to assess the level of knowledge, attitude and practice of faculty members and students of Melaka Manipal Medical College towards management of trauma to the anterior teeth.

Out of all 200 participants, almost 40% of them reportedly had witnessed or experienced dental trauma and the most common type of trauma experienced was tooth fracture. Those who had experienced dental trauma also had better knowledge score compared to those who have not experienced or witnessed any kind of dental trauma (P=0.09). Collectively, more than 80% of study participants did not have any previous knowledge on dental first aid. The results are similar to the study done by Mohandas et al, where 90% of the physical education teachers had received first aid training but among that 90%, only 4% had come across management of dental trauma as part of their first aid training. Even though only a few of the participants had previous knowledge about dental trauma, they showed better knowledge score with (P=0.025) compared to their counterpart.

While assessing practices among study participants, option ‘pick up the tooth, place in milk and go to dentist’ was chosen by as much as 18% of the study participants since, milk is proven to maintain the viable integrity and proliferative capability of periodontal ligaments cells. The next question regarding this case was about ways to arrest bleeding and almost 80% of study participants opted the option ‘ask child to bite on cotton pad’. The third question shows the highest percentage of the most proper answer whereby around 90% of study participants shows the urgency to bring the injured boy to the dentist immediately within 1 hour to manage the trauma, and for the last question, the study participants were asked about the consequence of not managing the dental injury properly and 50% answered ‘disrupts the eruption of permanent tooth’. 76% of study participants answered that they would immediately go to the dentist, and this shows their urgency on managing this matter similarly as in the first case. Even though fracture of enamel of a permanent tooth does not need any emergency care, dental attention should still be given to prevent any pulpal infections. In regards to the retention of dental knowledge, it is seen that the knowledge level of study participants is satisfactory. Almost 50% knows that it is possible to reattach a fractured tooth and 65.5% knows that is also possible to re-implant an avulsed or fallen tooth. 81.5% of study participants answered correctly regarding the way to handle an avulsed tooth and that is by holding the crown portion of the tooth, unlike previous study conducted. This is because to ensure a successful replantation of the tooth, damage to the pulpal tissue needs to be avoided, as they will help in the regeneration process later.

Most of the study participants felt that they should learn more about dental first aid and it should be part
of the curriculum. We found that 60% of the study participants were not reluctant to perform dental first aid if they encounter such situations and the remaining 40% are reluctant to do it with the most cited reason of fear of causing further harm or injury to the patients.\textsuperscript{11} Majority of the faculty members and students self-rated their knowledge on dental first aid as average and lack of professional training was the common reason reported by the faculty members and students on why they rated themselves, which was somehow in tandem with the previous findings.\textsuperscript{12,13,14}

**Conclusion**

The present study highlights some key factors related to lack in consistent knowledge and practices towards management of anterior tooth trauma. In spite of which, the participants of current study i.e. both faculty and students carry pretty good amount of not only knowledge and attitude but also, practices. The reason stated are different with common being the non-standardized and uniform knowledge not being imparted in all dental colleges in India. Although, a further more multicentric surveys involving more number of participants from various other medical and dental college and universities might give better clarity on the roadblocks.

**Conflict of Interest** – There are no conflicts of interest to declare.

**Ethical Clearance** – IEC 190/2019. Kasturba Medical College, Manipal.

**Source of Funding** – Not applicable

**References**


Effect of Sweet Potato Anthocyanin (Ipomoea Batatas L.) on Levels of Follicle Stimulating Hormone and Folliculogenesis in Rattus Norvegicus Exposed to Cigarette Smoke

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Abstract

Cigarette smoke is one of the exogenous free radicals that can enter the blood circulation so that it can disrupt all body cells and tissues, including the reproductive organs. The anthocyanin in purple sweet potato is one of the bioactive that can counter free radicals. This study was conducted to prove the effect of anthocyanin in purple sweet potato from Gunung Kawi cultivar on follicle-stimulating hormone (FSH), and folliculogenesis levels on Wistar rats (Rattus norvegicus) exposed to cigarette smoke. This study was an experimental study with Randomized Post Test Only Control Group Design, using 30 female Wistar rats aged 1-2 months weighing 150-200g. The rats were divided into three groups of where they were exposed to cigarette smoke and administered with anthocyanin (each group with the doses of 20, 40 and 80 mg/kgbw/day). One positive control group was exposed to cigarette smoke without being administered with anthocyanin for 56 days, and one more group served as a negative control. The FSH levels in the serum were measured by employing the ELISA method, and the folliculogenesis (follicle amount and number of ovary follicular granulosa cells) was measured from histopathological slides with Haematoxylin-Eosin staining. The results indicated that anthocyanin in purple sweet potato significantly increased the FSH levels, the follicle amount and the number of primary follicular granulosa cell, secondary, and Graafian on female Wistar rat ovaries exposed to cigarette smoke with a p-value of less than 0.05. To ensure the anthocyanin dose that had maximum effect, more research is required with more dose variations and chronic toxicity tests for the safety of the anthocyanin in purple sweet potato of Gunung Kawi cultivar.

Keywords: anthocyanin, purple sweet potato, Gunung Kawi cultivar, FSH, ovary, folliculogenesis

Introduction

Cigarette smoke is one of the exogenous free radicals that can enter the bloodstream so that it can disrupt all body cells and tissues. The body physiologically produces antioxidants to counter the reactivity of free radicals. This antioxidant captures the free radicals and prevents its reactivity amplification by cutting the free radical’s chain oxidation reactions with cellular components, causing this antioxidant to earn its name as the free radical scavenger. If the number of free radicals exceeds the amount of antioxidant in the body, free radicals will increase the ROS in the blood. If this condition continues without any resistance from the body, oxidative stress will take place.

Oxidative stress can cause synthesis and secretion disturbances on hypothalamic GnRH. This failure will cause the pituitary gland to fail the synthesis and secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). In addition, oxidative stress caused by cigarette smoke can affect folliculogenesis by inhibiting the follicle growth, increasing apoptosis, decreasing ovary volume and follicle count, and causing damage to the ovary, including granulosa cell degeneration. The cigarette smoke component that causes oxidative stress can cause DNA impairment to the follicles in the ovary, which is the source of estrogen hormone. Ganoon (2012) also suggests that nicotine suppresses follicle growth in the ovary, which results in the decreased levels of estrogen hormone.
One of the bioactive that can be used as antioxidants to counteract free radicals is anthocyanin. Anthocyanin is a potential antioxidant because of its capability to rapidly reduce oxygen species and turn it into a more stable aryloxyl radicals. This notion is supported by the research suggesting that anthocyanin can have higher antioxidant activity than vitamin E, vitamin C and beta-carotene. Zhao (2013) also reveals that the antioxidant effects of anthocyanin extract of sweet potato purple are higher than vitamin C.

Based on the background mentioned above, this study aims to prove the effect of anthocyanin in purple sweet potato (Ipomoea batatas L.) of Gunung Kawi cultivar on FSH serum and folliculogenesis levels on Wistar rats (Rattus norvegicus) exposed to cigarette smoke.

Materials and Methods

Experiment Animals

This study utilized healthy female Wistar rats and it was acclimatized. The samples of thirty rats aged 1-2 months, weighing 150-200g were divided into five groups, each has six rats, including one negative control group (without cigarette smoke exposure with anthocyanin), one positive control group (exposed to cigarette smoke without anthocyanin) and three treatment groups. The three treatment groups were the groups administered with anthocyanin at the doses of 20 mg/kg BW, 40 mg/kg BW, and 80mg/kg BW per day given through feeding tubes for eight weeks. All animals from the five groups were fed ad libitum.

Before starting the cigarette smoke exposure and anthocyanin administration, vaginal swabs were performed to see the estrus cycle. The cigarette smoke exposure started when the rats were in the proestrus phase. After eight weeks of exposure, vaginal swabs were performed on day 56 to determine the proestrus phase. The dissected rats were in the proestrus phase. The experimental animals were anesthetized through intramuscular injection (IM) in the thigh using 1% ketamine at a dose of 0.2ml.

Cigarette smoke exposure

Cigarette smoke exposure was administered as much as two sticks/day, i.e. one stick in the morning and one stick in the evening for eight weeks after they were found in the proestrus phase. The Cigarette smoke exposure box was made of fiberglass sized 26x12x12. It was only filled with three rats because there were only three rooms available in the smoking pump. The brand of cigarettes used was Gudang Garam Merah clove cigarettes (kretek cigarettes). After every exposure, the box was always cleaned from the remaining cigarette smoke from the previous treatment.

Anthocyanin Administration

Anthocyanin was administered by calculating the prescribed doses, which were 20mg/kgbw (Anthocyanin 1), 40 mg/kgbw (Anthocyanin 2), and 80mg/kgbw (Anthocyanin 3) daily. All doses were diluted using 1 ml of aquadest and were administered for one week. The anthocyanin solution was administered by using a 1ml syringe and then put into the rats’ stomach using feeding needles.

Sample Collection

After eight weeks of administration, the female Wistar rats were treated with vaginal swabs to determine the proestrus phase after dissection. Furthermore, the blood was taken intracardially through the right heart ventricle as much as 3 ml through injection syringe. Then, the blood was put in test tubes without administered with anticoagulant which was then covered with rubber plugs. Next, the left ovary organ was taken, then put into 10% formalin buffer solution.

FSH Level Examination by using ELISA method

The blood serum samples were placed into 50 µl microplates. Then, 100µl Enzyme Conjugate was added for each microplate, then shaken for 2-5 minutes. The microplates were then incubated at 37ºC for two hours. After incubation, the solution in the microplates was cleaned up. Then, the microplates were washed by using 300µl washing solution and then shaken for 3 minutes. The washing was repeated for five times. When finished, the plates were turned over, pressed firmly with absorbent paper, and dried using a tissue. Then, 100µl TBM substrate solution was added to each microplate in order. On the next step, the tubes were incubated for 20 minutes at a closed room temperature by using window film and then wrapped with aluminum foil.
foil. Next, the reaction was stopped by adding 50µl stop solution into each microplate gently. Then, they were shaken for 5 seconds. The FSH level examination was conducted by inserting the microplates into the ELISA Spectrophotometer. The results were read at a wavelength of 450nm.

**Making of HE Ovary Preparation**

The ovary tissue was dissected to a thickness of 2-3 millimeters and then put into a 10% fixative formalin buffer. The tissue was then cut into 3-5 µm thickness for histopathological examination using Hematoxylin-Eosin (HE) staining.

**Examination of Ovary Follicle Number**

The number of ovary follicles was calculated from the ovary histopathological slides by using the Olympus XC 10 Dotslide Microscope for the overall cross-section and further identified with 400 magnification to calculate the primary, secondary and Graafian follicle.

**Examination of Ovary Follicular Granulosa Cells**

The calculation of the number of primary, secondary and Graafian follicular granulosa cells was carried out after obtaining the overall number of follicles. Then, the slides were explored to determine the examination visual fields of the primary follicular granulosa cells (4 visual fields), secondary follicular granulosa cells (5 visual fields), and Graafian follicular granulosa cells (5 visual fields) by using Olympus XC 10 Dotslide Microscope with 1,000 times magnification.

**Statistic Analysis**

The research results are expressed as average ± standard deviation (SD) of the mean. The data on FSH levels, the number of primary, secondary, and Graafian follicles, and the number of primary and secondary follicular granulosa cells were tested using One Way ANOVA difference test. Then the assessment proceeded with post-hoc LSD (Least Square Differences) test, while the number of Graafian follicular granulosa cells were calculated using Kruskal-Wallis test and further proceeded with the Mann Whitney-U test using SPSS version 20.0 (SPSS Inc., IBM). The coincidence interval was set at 95% and declared significant if the p-value was less than 0.05.

**Results**

The Effect of cigarette smoke exposure on FSH levels and folliculogenesis in female white rats (*Rattus Norvegicus*)

The difference test results in cigarette smoke exposure towards the FSH levels and folliculogenesis were carried out by comparing the negative control group with the positive control group.

**Table 1. The Effect of cigarette smoke exposure on FSH levels and folliculogenesis in female Wistar rats (Rattus Norvegicus)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative control</th>
<th>Positive control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg ± stand.dev</td>
<td>Avg ± stand.dev</td>
<td></td>
</tr>
<tr>
<td>FSH level (miU/ml)</td>
<td>3.69±0.67</td>
<td>2.46±0.82</td>
<td>0.017&lt;α</td>
</tr>
<tr>
<td>Primary follicles count</td>
<td>7.17±1.17</td>
<td>4.67±0.82</td>
<td>0.002&lt;α</td>
</tr>
<tr>
<td>Secondary follicles count</td>
<td>8.33±1.21</td>
<td>4.50±1.05</td>
<td>0.000&lt;α</td>
</tr>
<tr>
<td>Graafian follicles count</td>
<td>3.33±1.21</td>
<td>0.83±0.75</td>
<td>0.002&lt;α</td>
</tr>
<tr>
<td>Primary follicular granulosa cells count</td>
<td>20.83±3.07</td>
<td>13.79±2.51</td>
<td>0.001&lt;α</td>
</tr>
<tr>
<td>Secondary follicular granulosa cells count</td>
<td>81.37±5.12</td>
<td>57.17±5.89</td>
<td>0.000&lt;α</td>
</tr>
<tr>
<td>Graafian follicular granulosa cells count</td>
<td>86.57±3.31</td>
<td>66.60±32.65</td>
<td>0.005&lt;α</td>
</tr>
</tbody>
</table>

(Rattus Norvegicus)
The effect of anthocyanin in purple sweet potato (*Ipomoea batatas* L.) of Gunung Kawi cultivar on FSH levels in female Wistar rats (*Rattus Norvegicus*) exposed to cigarette smoke

The histogram in Figure 1 indicates a significant difference in the FSH levels of the rats in Anthocyanin 2, and Anthocyanin 3 treatment groups in comparison to the positive control group, with a p-value of 0.020.

![Figure 1. FSH serum level in all groups](image)

Negative Control: rats without cigarette smoke and anthocyanin exposure

Positive controls: rats exposed to cigarette smoke but without anthocyanin

Anthocyanin 1: with a dose of 20 mg/kgbw in rats exposed to cigarette smoke

Anthocyanin 2: with a dose of 40 mg/kgbw in rats exposed to cigarette smoke

Anthocyanin 3: with a dose of 80 mg/kgbw in rats exposed to cigarette smoke

The Effect of anthocyanin in purple sweet potato (*Ipomoea batatas* L. anthocyanins) of on the ovary follicles in female Wistar rats (*Rattus Norvegicus*) exposed to cigarette smoke

The administration of anthocyanin from the purple sweet potato of Gunung Kawi cultivar significantly increased the number of primary, secondary and Graafian follicles compared to the positive control group (p-value <0.05), the mean and standard deviation of follicle counts are indicated in Table 2.
Tabel 2. The Effect of anthocyanin in purple sweet potato (*Ipomoea batatas* L. *anthocyanins*) of Gunung Kawi cultivar on the ovary follicles in female Wistar rats (*Rattus Norvegicus*) exposed to cigarette smoke

<table>
<thead>
<tr>
<th>Treated Groups</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Anthocyanin 1</th>
<th>Anthocyanin 2</th>
<th>Anthocyanin 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>7.17±1.17 a</td>
<td>4.67±0.82 b</td>
<td>5.17±0.75b</td>
<td>6.67±1.37a</td>
<td>7.50±1.05a</td>
</tr>
<tr>
<td>Secondary</td>
<td>8.33±1.21 a</td>
<td>4.50±1.05 b</td>
<td>4.83±0.75b</td>
<td>6.17±1.94b</td>
<td>7.67±1.03a</td>
</tr>
<tr>
<td>Graafian</td>
<td>3.33±1.21 a</td>
<td>0.83±0.75 b</td>
<td>1.33±0.82bc</td>
<td>2.50±1.05ac</td>
<td>2.83±1.33ac</td>
</tr>
</tbody>
</table>

Information: the value indicates an average rate ± of SD with *p*-value <0.05

Negative Control: rats without cigarette smoke and anthocyanin exposure,

Positive controls: rats exposed to cigarette smoke but without anthocyanin

Anthocyanin 1: with a dose of 20 mg/kgbw in rats exposed to cigarette smoke

Anthocyanin 2: with a dose of 40 mg/kgbw in rats exposed to cigarette smoke

Anthocyanin 3: with a dose of 80 mg/kgbw in rats exposed to cigarette smoke

The Effect of anthocyanin in purple sweet potato (*Ipomoea batatas* L. *anthocyanins*) of Gunung Kawi cultivar on the number of ovary follicular granulosa cells in female Wistar rats (*Rattus Norvegicus*) exposed to cigarette smoke

The administration of anthocyanin in purple sweet potato of Gunung Kawi cultivar significantly increased the number of primary, secondary and Graafian follicular granulosa cells compared to the positive control group (*p*-value <0.05). The mean and standard deviation of the follicular granulosa cells is presented in Table 3.

Table 3. The Effect of anthocyanin in purple sweet potato (*Ipomoea batatas* L. *anthocyanins*) of Gunung Kawi cultivar on the number of ovary follicular granulosa cells in female Wistar rats (*Rattus Norvegicus*) exposed to cigarette smoke

<table>
<thead>
<tr>
<th>Treated Groups</th>
<th>Negative Control Group</th>
<th>Positive Control Group</th>
<th>Anthocyanin 1</th>
<th>Anthocyanin 2</th>
<th>Anthocyanin 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>20.83±3.07a</td>
<td>13.79±2.51b</td>
<td>13.96±2.46b</td>
<td>18.79±1.82a</td>
<td>22.33±0.54a</td>
</tr>
<tr>
<td>Secondary</td>
<td>81.37±5.12a</td>
<td>57.17±5.90b</td>
<td>60.73±4.34b</td>
<td>74.20±3.88c</td>
<td>81.60±5.63a</td>
</tr>
<tr>
<td>Graafian</td>
<td>86.57±3.31a</td>
<td>53.27±41.35b</td>
<td>69.13±33.88bc</td>
<td>82.73±1.37ac</td>
<td>86.90±2.44a</td>
</tr>
</tbody>
</table>

Information: the value indicates an average rate ± of SD with *p*-value <0.05
Negative Control: rats without cigarette smoke and anthocyanin exposure

Positive controls: rats exposed to cigarette smoke but without anthocyanin

Anthocyanin 1: with a dose of 20 mg/kgbw in rats exposed to cigarette smoke

Anthocyanin 2: with a dose of 40 mg/kgbw in rats exposed to cigarette smoke

Anthocyanin 3: with a dose of 80 mg/kgbw in rats exposed to cigarette smoke

Discussion

Cigarette smoke is an exogenous free radical that can accumulate in female reproductive organs\(^{13}\). The effects of free radicals on the reproductive organs include disorders of reproductive hormones, ovary maturation, ovulation, follicle development, fertilization and implantation.

In this study, it was found that cigarette smoke exposure on female Wistar rats significantly decreased the FSH levels and folliculogenesis in comparison to the control group (p-value <0.05). This indicates that cigarette smoke contains free radicals which can cause an increase in oxidative stress, either directly or indirectly\(^{14}\). Because of the increased oxidative stress, lipid peroxide occurs which can cause damage to the arcuate nucleus and ventromedial nucleus in the hypothalamus, resulting in disruption of hypothalamic GnRH synthesis and secretion. This failure will cause pituitary failure to synthesize and secrete FSH and LH. The excessive amount of cigarette smoke entering the body through inhalation will cause oxidative stress in the brain, resulting in brain cell degeneration which will cause damage to the hypothalamus. If this stage occurs, the levels of GnRH (gonadotropin releasing hormone) will decrease and will have an impact on the decreasing levels of FSH (follicle stimulating hormone) and LH (luteinizing hormone). This level decrease of FSH and LH hormones will interfere with the development of granulosa cells and the number of ovary follicles. This will disrupt ovary function and can result in infertility\(^{4,14}\).

The administration of anthocyanin from the purple sweet potato of Gunung Kawi cultivar with several dose levels in this study has indicated that there was an increase in FSH levels compared to the positive control group. At 80mg/kgbw anthocyanin dose, the highest FSH level was obtained compared to the other doses. From the study results, it was found that the fastest dose of anthocyanin in purple sweet potato from Gunung Kawi to increase FSH serum levels, follicle counts and the number of primary, secondary and Graafian follicular granulosa cells in female Wistar rats exposed to cigarette smoke was 80mg/kgbw (A3 treatment) in comparison to the anthocyanin doses of 20 mg/kgbw and 40 mg/kgbw. This proves the theory stating that anthocyanin can act as antioxidants. Anthocyanin is neuroprotective because it can capture ROS and prevent the occurrence of lipid peroxidation processes in the brain. The absence of oxidative stress in the brain will have an impact on increasing sex hormones (FSH and LH)\(^{15}\). Anthocyanin functions as an antioxidant by reducing free radicals, donating electrons to free radicals\(^{16,17}\). In addition, the anthocyanin from purple sweet potato can increase the Total Antioxidation Capacity (T-AOC), i.e. the enzymatic antioxidants in the body (SOD and GSH-PX) and reduce MDA levels so that the oxidative stress does not occur in body organs, including the ovary. This condition will affect the process of follicle and granulosa cell development\(^{12}\).

This study concludes that the anthocyanin in purple sweet potato of Gunung Kawi cultivar can increase FSH levels and folliculogenesis in female white rats exposed to cigarette smoke. However, this study cannot be generalized to humans because there has not been a chronic toxicity test and the dose of anthocyanin from purple sweet potato has not been found to be truly optimal for all organs instead of only the reproductive organs.

Conclusion

The results indicated that anthocyanin in purple sweet potato significantly increased the FSH levels, the follicle amount and the number of primary follicular granulosa cell, secondary, and Graafian on female Wistar rat ovaries exposed to cigarette smoke.

Ethical Clearance

The ethics research from Ethic Committee of the Faculty of Medicine, University of Brawijaya, Malang
with number No. 274/EC/KEPK/07/2016.

Conflict of Interest: None

Source of Funding: The research was funded by the authors

References

Correlation between the Expression of E6 HPV with p53 and p16INK4A at Cervical Adenocarcinoma and Cervical Normal

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Abstract

Introduction: Cervical adenocarcinoma is the second most common type of cervical cancer. The marker to predict the risk of this cancer is still need to be improved. This study aimed to evaluate the correlation between expression of E6 HPV with p53 and p16INK4A in cervical adenocarcinoma and cervical normal.

Methods: This study was a cross sectional study conducted at the Department of Anatomic Pathology, Dr. Soetomo General Academic Hospital Surabaya Indonesia using 49 paraffin blocks during period January-December 2017. The E6 HPV, p53, and p16INK4A expression were detected by immunohistochemistry staining.

Results: There were significant differences between the expression of E6 HPV and p16INK4A in cervical adenocarcinoma and cervical normal, but the expression of p53 was no significant difference with p=0.060 (P>0.05). There was a positive correlation between the expression of E6 HPV with p53 and p16INK4A expression (p<0.05) in cervical adenocarcinoma and cervical normal, and in cervical adenocarcinoma group, there was a significant correlation between the expression of E6 HPV with p53 (p=0.004) and p16INK4A (p=0.028), but no correlation was found between p53 with p16INK4A expression (p=0.092).

Conclusion: It suggests that p16INK4A might serve as marker for predicting the risk of developing cervical cancer from the epithelium of cervical normal.

Keywords: cervical adenocarcinoma, cervical normal, E6 HPV, p53, p16INK4A

Introduction

Worldwide, cervical cancer is the third most common cancer ranking after breast and colorectal, with an estimated 530,000 new cases and 275,000 deaths in 2008. It is the fourth most common cause of cancer death ranking below breast, lung, and colorectal cancer. This is estimated that incidence and mortality rate of 15 and 8 per 100,000 respectively¹. Indonesia has a population of 132,521,684 women and is at great risk for cervical cancer. In 2018, cervical cancer is the second most common cancer after breast cancer, with 32,469 new cases and 18,279 death². The most common type of cervical cancer is squamous cell carcinoma, which affects ectocervical part, and the second most common type, is cervical adenocarcinoma, which affects the endocervical part, with an incidence of about 15% and the incidence tends to increase³.

The cause of cervical cancer is infection of Human Papillomavirus (HPV). The HPV virus infects the cervix through sexual contact, causing cervical squamous cell carcinoma and cervical adenocarcinoma⁴. The HPV 16 and 18 cause the 70% of cervical cancer in the world⁵, while HPV 6 and 11 cause the 90% of anogenital wart cases⁶. The HPV viral oncogenes, E6 and E7, have shown to be the main contributors to the development of HPV-induced cancers. These oncogenes have the ability to bind host cell regulatory proteins, especially tumor suppressor gene products. The HPV E6 protein
complexes with cellular proteins E6-AP and p53 and facilitates p53 degradation via the ubiquitin-dependent proteolytic system. E6 proteins of both high risk and low risk HPV types bind to p53 in vitro, but only E6 proteins of oncogenic HPV types can target p53 for degradation, thus inhibits the stabilization and activation of p53\textsuperscript{11}. The HPV oncoprotein E7 is known to bind and inactivate hypophosphorylated retinoblastoma protein (pRB), which eventually leads to upregulation of p16INK4A. P16INK4A is a tumor suppressor protein that inhibits cyclin dependent kinases (CDK)-4 or -6 binding to cyclin D which regulates the G1 cell cycle checkpoints\textsuperscript{12}.

Both p53 and p16INK4A in normal cell is almost undetectable by IHC staining. The expression of wild-type p53 (WT) has a very short half-life\textsuperscript{13}, therefore it is undetectable by immunohistochemistry (IHC) staining. In HPV infection cases, E6 protein of HPV will degrade p53 protein. p16INK4A protein in normal cells is expressed in very low level and almost undetectable by IHC. Because of the transforming activity of E7 oncogene, p16INK4A is strongly expressed in tumor cells affected by HPV and may be easily detected by IHC\textsuperscript{14}. However, the correlation between E6, p53, and p16 do not clear yet. Hence, the objective of this study was to evaluate the correlation between the expression of oncoprotein of E6 HPV with the expression of p53 and p16INK4A in cervical adenocarcinoma and cervical normal.

**Material and Method**

This study was a cross sectional study conducted at the Department of Anatomic Pathology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia during period January-December 2017. The study was approved by Ethics Committee of the Faculty of Medicine, Universitas Airlangga. The specimens of this study were 10 formalin fix paraffin embedded (FFPE) specimens of cervical normal from uterine prolapse cases and 39 FFPE of cervical adenocarcinoma cases. The diagnosis of cervical adenocarcinoma was made by a pathologist. The selection of FFPE samples using purposive random sampling, with inclusion criteria were specimens diagnosed as cervical adenocarcinoma histopathologically and tumor tissue in FFPE still has enough for IHC staining. The Pathologists sort and adjust between representative slides of cervical adenocarcinoma tissue and paraffin blocks.

E6 HPV was detected by immunohistochemistry (IHC) staining using the HPV16 E6/18 E6 Antibody (C1P5): SC-460 (Santa Cruz Biotechnology). Positive interpretation of the IHC staining was based on nuclear or combined nuclear and cytoplasmic staining. Only cytoplasmic staining was considered as negative\textsuperscript{15}. Scoring of the E6 HPV 16/18 was performed using an arbitrary semi quantitative scale. There was no staining that represented negative staining (score 0), 5-25% staining represented mild positive staining (score 1), 25-50% staining represented moderate positive staining (score 2), and >50% represented extensive positive staining (score 3)\textsuperscript{16}.

The expression of p53 was detected by IHC staining using monoclonal antibody p53 clone Y5 (Biocare). p53 is regarded as having score 0 if 0-5% of the cells were positive, score 1 if 5-25% of the cells were positive, score 2 if 26-50% were positive, score 3 if 51-75% were positive, and score 4 if >75% of the cells were positive\textsuperscript{17}.

p16INK4A expression was detected by IHC staining using the Anti-CDKN2A/p16INK4a Antibody (clone 1E12E10) IHC-plus™ LS-B5261 (LS Bio). Scoring of p16INK4A was negative (score 0) if <1% of the cells were positive, sporadic (score 1) if 1-5% of the cells were positive, focal (score 2) if 5-25% of the cells were positive, and diffuse (score 3) if >25% of the cells were positive\textsuperscript{18}.

Statistical analysis to evaluate the differences between the expression of E6 HPV, p53 and p16INK4A at cervical normal tissue and adenocarcinoma tissue was performed using The Kruskal-wallis (p<0.005) test and its correlation was analyzed by Spearman correlation test (p<0.005).

**Result**

This study used cervix specimen of women aged 30-81 years with mean ± SD of 51.78 ± 9.395. Cervical normal tissue was found in 10 specimens (20.4%) and cervical adenocarcinoma tissue were in 39 specimens (79.6%) (Table 1).
Table 1. Ages rank of the patient of cervical normal and cervical adenocarcinoma

<table>
<thead>
<tr>
<th>Ages</th>
<th>The number of persons</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>40-49</td>
<td>25</td>
<td>51.0</td>
</tr>
<tr>
<td>50-59</td>
<td>13</td>
<td>26.5</td>
</tr>
<tr>
<td>60-69</td>
<td>9</td>
<td>18.4</td>
</tr>
<tr>
<td>70-79</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>80-89</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>99.9</td>
</tr>
</tbody>
</table>

The results showed positivity and negativity for IHC staining of E6 HPV, p53, and p16INK4A expression in cervical normal and adenocarcinoma (Figure 1).

Figure 1. The immunohistochemistry staining of the E6 HPV, p53, and p16INK4A expression in cervical normal and adenocarcinoma. The expression of the E6 HPV negative staining in cervical normal (A) and positive staining in cervical adenocarcinoma 400 x (B). The expression of p53 negative staining in cervical normal (C) and positive staining in cervical adenocarcinoma 400x (D). The expression of p16INK4A negative staining in cervical normal (E) and positive staining in cervical adenocarcinoma 400x (F).
The E6 HPV expression in all of the cervical normal were negative, whereas in cervical adenocarcinoma the expression was positive with scores 0, 1, 2, and 3. We found significant difference between the expression of E6 HPV in cervical normal and in cervical adenocarcinoma with p=0.001 (P<0.05) (Table 2). Profiles of p53 expression in cervical normal showed that 60% (6/10) had score 0 and 40% (4/10) had scores 1 and 2, whereas in cervical adenocarcinoma the scores varied between 0, 1, 2, 3, and 4, but there was no significant difference with p value=0.060 (P>0.05) (Table 2). Profile of p16INK4A expression in cervical normal showed that 90% (9/10) had score 0 and 10% (1/10) had score 1, whereas in all cervical adenocarcinoma tissues had scores of 1, 2, and 3, and most of the specimens had score 3. Statistical analysis showed that there was a significant difference between p16INK4A expression in adenocarcinoma and cervical normal with p<0.0001 (P<0.05) (Table 2).

Table 2. The profile of E6 HPV, p53, and p16INK4A expression in cervical normal and cervical adenocarcinoma

<table>
<thead>
<tr>
<th>Score</th>
<th>Cervical Normal</th>
<th>Cervical Adenocarcinoma</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6 HPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10 (100%)</td>
<td>14 (35.9%)</td>
<td>24 (49%)</td>
<td>0.001</td>
</tr>
<tr>
<td>1</td>
<td>0 (0%)</td>
<td>11 (28.2%)</td>
<td>11 (22.4%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0 (0%)</td>
<td>11 (28.2%)</td>
<td>11 (22.4%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0 (0%)</td>
<td>3 (7.7%)</td>
<td>3 (6.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6 (60%)</td>
<td>12 (30.8%)</td>
<td>18 (36.7%)</td>
<td>0.060</td>
</tr>
<tr>
<td>1</td>
<td>3 (30%)</td>
<td>15 (38.5%)</td>
<td>18 (36.7%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 (10%)</td>
<td>3 (7.7%)</td>
<td>4 (8.2%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0 (0%)</td>
<td>4 (10.3%)</td>
<td>4 (8.2%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0 (0%)</td>
<td>5 (12.8%)</td>
<td>5 (10.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p16INK4A</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>0</td>
<td>9 (90%)</td>
<td>0 (0%)</td>
<td>9 (18.4%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (10%)</td>
<td>1 (2.6%)</td>
<td>2 (4.1%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0 (0%)</td>
<td>3 (7.7%)</td>
<td>3 (6.1%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0 (0%)</td>
<td>35 (89.7%)</td>
<td>35 (71.4%)</td>
<td></td>
</tr>
</tbody>
</table>
There was a significant correlation between the expression of E6 HPV with the expression of p53 and p16INK4A (p<0.05) from all of data both cervical adenocarcinoma and normal. In cervical adenocarcinoma group, there was a significant correlation between the expression of E6 HPV with the expression of p53 and p16INK4A, but in cervical normal, the data could not be analyzed because it was constant (Table 3). The Spearman’s coefficient (rs) for expression of E6 HPV and p53 from cervical adenocarcinoma was 0.402 with p value 0.004 (p<0.05). We found significant positive correlation between the expression of E6 HPV with p53 (Table 3). The rs for expression of E6 HPV with p16INK4A was 0.313 with p value of 0.028 (p<0.05), indicating a positive significant correlation between the expression of E6 HPV with p16INK4A in cervical adenocarcinoma (Table 3). These results indicate that the increase in the expression of E6 HPV is along with increased expression of p16 protein. There was no correlation between p53 expression and p16INK4A with Spearman’s coefficient rs 0.244 with p value=0.092 (p>0.05 (Table 3).

### Table 3. The Spearman correlation test of the expression of E6 HPV with the expression of p53 and p16INK4A

<table>
<thead>
<tr>
<th></th>
<th>ADC (n=39)</th>
<th>CN (n=10)</th>
<th>Total (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6 HPV with p53</td>
<td>0.352</td>
<td>-</td>
<td>0.402</td>
</tr>
<tr>
<td>P value</td>
<td>0.028</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>E6 HPV with p16INK4A</td>
<td>-0.262</td>
<td>-</td>
<td>0.313</td>
</tr>
<tr>
<td>P value</td>
<td>0.107</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>p53 with p16INK4A</td>
<td>0.034</td>
<td>-0.266</td>
<td>0.244</td>
</tr>
<tr>
<td>P value</td>
<td>0.835</td>
<td>0.458</td>
<td>0.092</td>
</tr>
</tbody>
</table>

Note: ADC= cervical adenocarcinoma, CN= cervical normal

### Discussion

Cervical adenocarcinoma is one of the most frequent epithelial malignancies of the cervix after a squamous cell carcinoma. Screening for this type of adenocarcinoma cancer is more difficult because it is located in the endocervical canal. Therefore, the patient is often diagnosed at an advanced stage.

This study used the cervical normal tissue as control group and cervical adenocarcinoma tissue. The expression of E6 HPV in the study was a parameter for detecting the infection of HPV 16/18. Positive IHC staining showed that it was infected by HPV 16 and or 18 on the tissue. The expression of p53 in this study was a parameter to determine p53 activity in cervical adenocarcinoma tissue and cervical normal as control. p16INK4A expression in this study was a parameter for detecting p16INK4A protein accumulation in the tissues.

This study showed that E6 HPV in cervical normal was negative, while in cervical adenocarcinoma, it was both negative and positive. These suggested that in
cervical normal tissue, there is no expression of E6 HPV 16/18 oncoprotein. This indicated that cervical normal tissue in this study was not infected with HPV 16 and or 18. This study also showed that there was a significant difference in the expression of E6 HPV between cervical normal and adenocarcinoma tissue. This indicates that in adenocarcinoma cervical tissue there is an infection of HPV 16 and or 18. This is in accordance with the results of previous research that the HPV virus infects the cervix in the ectocervical area, causing squamous cell carcinoma and cervical adenocarcinoma. The genotype of HPV 16 and 18 cause for 70% of cervical cancer in the world, while HPV 6 and 11 account for 90% of the causes of anogenital warts.

HPV E6 protein binds to p53 and stimulated p53 degradation through a binding mechanism called ubiquitin-dependent proteolytic pathway (E6AP) to produce ubiquitination p53. Positive results for p53 in IHC staining may indicate accumulation of mutant-type p53 or ubiquitinated form of p53 undergoing degradation, but it still needs further study. Overexpression of p53 suggested accumulation of p16INK4A because pRb normally inhibits p16INK4A transcription. Increasing expression of the viral oncogenes in dysplastic cervical cells might thus be reflected by increased expression of p16INK4A. This study showed that cervical normal tissues were IHC staining p16INK4A is physiologically expressed very low in some cells, especially cells undergoing squamous metaplasia process). In contrast, due to the activity of cell transformation due to the oncogenic E7 HPV High Risk (HR) protein, p16INK4A is expressed very strongly in cervical cells with dysplasia and may easily be detected by IHC. Therefore, p16INK4A may be considered as a surrogate marker for HPV HR oncogene expression. In the epithelium of cervical normal, it was negative for 90% (9/10) specimens and was positive for 10% (1/10) specimens in sporadic appearance. It is also reported that 10% of cervical normal specimens expressed p16INK4A. This study showed that there was a significant difference between p16INK4A protein expression in cervical adenocarcinoma and cervical normal. This finding suggested that it might serve as a marker for predicting the risk of developing cervical cancer in epithelium of cervical normal and the IHC of p16INK4A might be used as supplementary test for cervical specimens.

HPV is an etiological agent of cervical cancer and classified into high-risk and low-risk genotypes according to their association with cancer. The oncogenic proteins are E6 and E7, E6 high risk HPV promote the degradation of p53, while E6 low risk. Expression of the E6 protein from HPV high risk resulted in degradation of p53, whereas E6 from HPV low risk did not. The E7 HPV inactivates pRB. There was a correlation between the expression of p16INK4A and pRb in cervical neoplasia. pRB inhibits the transcription of the cyclin-dependent kinase inhibitor gene p16INK4A. Increasing expression of the viral oncogenes in dysplastic cervical cells might thus be reflected by increased expression of p16INK4A. This study showed positive correlation between the expression of E6 HPV 16/18 with the expression of p53 and p16INK4A in cervical adenocarcinoma. Another study also showed an association between p16INK4A and endocervical carcinomas. This indicate that p16INK4A is a specific marker for premalignant and malignant lesions of the squamous and endocervical mucosa and it may be used as an important tool for reducing incorrect diagnosis of adenocarcinoma. However, the expression of E6 HPV 16/18 and p16INK4A between cervical normal and cervical adenocarcinoma were significantly different, while the expression of p53 was not significantly different. It showed that cervical normal tissues were
not infected by HPV 16/18, so that it resulted in no expression of p16INK4A. Therefore, we suggest that pathology centers in Indonesia, where HPV examination cannot be performed, might perform IHC for p16INK4A as a substitute method of examination.

**Conclusion**

In conclusion, this study showed positive correlation between the expression of E6 HPV 16/18 with p53 and p16INK4A in cervical normal and cervical adenocarcinoma tissues. We also found significant different expression of E6 HPV 16/18 and p16INK4A between cervical normal and cervical adenocarcinoma tissues. It suggested that p16INK4A might serve as marker for predicting risk of developing cervical cancer in epithelium of cervical normal and the IHC of E6 HPV and p16INK4A may be used as supplementary test for cervical specimens.

**Acknowledgment:** We thank to the Government of the Republic of Indonesia and the Faculty of Medicine, Universitas Airlangga for financial support in this study.

**Funding:** This study was supported by the Ministry of Research Technology and Higher Education of the Republic of Indonesia by the university operational funding.

**Conflict of Interest:** None

**Ethical Permission:** The study was approved by Ethics Committee of the Faculty of Medicine, Universitas Airlangga, using formalin fix paraffin embedded (FFPE) specimens.

**References**

Correlation of Physical Activity with Fear of Fall in Patients with Total Knee Replacement - A Research Protocol

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Wardha, Maharashtra

Abstract

Background: An elderly patient who has undergone total knee replacement may have fear of fall. Fear of fall after TKR and its impact on physical activity is sparsely researched. This study was aimed to find a correlation of physical activity with fear of fall in patients with Total Knee Replacement. Physical activity is assessed using the Physical activity scale for the elderly (PASE) Scale. The PASE Scale is understandable, reliable, and is a valid tool to assess physical activity in the elderly. Fall efficacy scale (FES) is a valid tool to assess fear of fall. Objectives: The objective of the current study is to assess physical activity and fear of fall and to find Correlation of physical activity with fear of fall in patients with TKR. Methods: This study will be carried out in Physiotherapy OPD, Ravi Nair Physiotherapy College and AVBRH Hospital Sawangi Meghe, Wardha. 100 Patients undergone TKR will be examined. Physical activity and fear of fall will be assessed using the PASE scale and Fall Efficacy Scale respectively. Results: Upon completion of the Study result will be estimated by statistical analysis. Conclusion: After going through various studies, maximum studies indicated that there is a decrease in physical activity in patients with TKR but very few studies indicated fear of fall in the patients. Therefore, the current study is being carried out which could help to reach the conclusion to find whether there is any correlation between physical activity and fear of fall in patients undergone TKR. Because rehabilitation after TKR is mainly concentrated on improving the physical function however if the patient has fear of fall this may interfere with the patient returning to his or her physical activity.

Keywords: TKR (Total Knee Replacement), Fear of fall, Physical activity, PASE Scale, FES, Elderly population

Introduction

The most common surgical procedure performed in patients with chronic knee arthritis is total knee replacement worldwide (¹). Deficits after Total Knee Replacement is seen for an extended period of 7 years or even more. This significantly affects the functional independence of the patients in terms of walking speed, postural stability, stair climbing this in turn reduces muscular strength, range of motion, and an altered pattern of motion that is visible postoperatively.

Balance is important to maintain postural stability while conducting functional activities and for avoiding fall. Stability (static and dynamic) is a complex process that involves the integration of sensory information about the body’s location and its ability to provide an effective motor response to the movement of the body. (²)

Various activities like active living regular exercises, ability to transportation are all included in a broad term as Physical Activity. (³⁵) These include household activities,
(3) Social participation, and recreational activities. After undergoing total knee replacement physical activity of the patient can be severely affected if the patient is not provided with appropriate rehabilitation. Furthermore, this may lead to reduced muscle strength and physical activity which will again affect the strength in the lower extremity. This vicious cycle will go on continuing to make the patient with total knee replacement physically inactive. This may result in loss of balance, reduced confidence, and make them dependent and may increase fear of fall.

Total knee replacement is mostly associated with osteoarthritis and physiological factors like weakness of muscle and impaired postural control may contribute towards fall in the elderly. The above-mentioned factors can further reduce the ability of the patient effectively resulting in low confidence and reduced quality of life.

(4) Balance is important for the maintenance of posture and for the avoidance of fall. Fall is described as an event that inadvertently leads a person to rest on the floor. Fear of falling is more complex, referring to people who trust in their own ability to perform a task safely without losing equilibrium. Some researchers have declared it as a loss of trust in patients’ ability to balance, other people have described the fear of falling in different ways to someone who involves avoiding daily activities and to another losing trust in balance and walking which may contribute to delayed recovery after total knee replacement. Few elderly people develop symptoms or Behaviour in response to a fall without any physical trauma. They can voice an increased or decreased fear of falling that can lead to emotional delivery, changes in psychology, or social changes. Fear of falling when Usually referred to as an adverse outcome of the fall, not much is known about it. It is possible to provide interventions to avoid fear and certain conditions caused due to it, if fear is the only factor that is responsible for physical inactivity.

(5) Counselling that should be provided to the patients after the surgery for total knee replacement by the health care workers in the developing world setting about physical activity is limited due to the insufficient data on the demands for physic activity and various activities that are being pursued, also the inherent socio-cultural differences, in a developing country physical activities following Total Knee Replacement that varies significantly from those performed by the patients who lives in a developed country. Specific life requirements can require a greater emphasis on daily activities, thus reducing time for discretional activities. Furthermore, rehabilitation can be affected because of not having enough access to physiotherapy and postoperative treatment until patients return home.

Knee osteoarthritis is an often-chronic condition that can cause joint pain, muscle weakness in lower limbs, and physical dysfunction in elder males and females. Apparently, The most successful operative treatment for chronic knee osteoarthritis is total knee replacement, since it relieves pain and improves mobility in around 80 percent of patients. Since replacement of knee decreases knee pain and enhances physical function patients will be able to increase their degree of physical activity but sometimes due to fear of falling the patient is not able to initiate the activity. Nevertheless, while total knee replacement patients show gains in their Physical activity twelve months after surgery, still they do not meet the required amount of physical activity. High rates of sport and intense work practices, on the other hand, have been identified as significant risk factors for early implant failure. Therefore, Physical activity assessment and fear of fall assessment using the valid, accurate, and reproducible tool are of particular concern for total knee replacement patients.

The Physical Activity Scale for Elderly (PASE) is a test that is created especially for adults aged sixty-five and over. This measures the strength, intensity, and duration of physical activity, which may be performed by self or interviewer. The benefits of the PASE scale is that it requires a limited time to finish, the time period for the retrieval is quick.

Moreover, persons who undergo total knee replacement are at risk for multiple problems, such as inflammation, periprosthetic fracture, symptomatic loss of implants. These complications significantly reduce the benefits of total knee replacement and often require revision surgery.

Total knee replacement(TKR) is intended to enhance function and decrease the pain that occurs because of osteoarthritis and should therefore enable these patients to increase their levels of physical activity after the
operation. But, there were contradictory reports on the rates of physical activity after total knee replacement.

Although some research indicated increased physical activity, others found little to no improvement in the average rate of physical activity after undergoing Total knee replacement but there is less evidence on the correlation of physical activity with fear of fall. Therefore, the current study focuses to correlate fear of fall and physical activity after total knee replacement.(10)

Patients with total knee replacement experience postoperative pain regularly. Post-operative exercise strategies are suggested but the effect of various physical activity is uncertain. Nevertheless, postoperative pain after total knee replacement frequently continues for several years. Postoperative pain interferes with healing, leading to delayed or poor recovery. Regulating physical activity is a kind of non-pharmacological treatment strategies for pain. Originally, the standard solution for treating acute skeletal and muscular pain was bed rest, but more recently, exercise was advised to enhance pain and quality of life as much as possible. Exercise did postoperative, which includes moving out of bed and walking as soon as possible after the procedure, is also recommended in the patients who have had surgery of Total knee replacement. Improved physical activity postoperatively is critical in functional recovery. Suitable physical activity pacing is then effective in minimizing the intensity of musculoskeletal pain. Physical activity pacing aims to remove the variability of ups and downs in the pattern of exercise, maintaining a consistent pace during the day, and preventing a blind increase in physical exercise. Poor physical activity gives rise to discomfort, psychological symptoms, and physical impairment in patients having a higher amount of pain. However, postoperative patients are also usually recommended only to increase physical activity as often as possible to enhance physical function postoperatively. The effect of variability in day-to-day physical activity has not been studied. Therefore, proper assessment of physical activity is needed. (11)

Falls impact about one-third of older adults residing in the city per year and have significant safety and social implications. Older people have the impact of Fear of falling (FOF) regardless of any experience of fall they have encountered previously. The health of the person is affected because of activity limitation which is due to fall and also fear of fall. There are lower rates of self-reported Physical activity likely because of lack of movement after injury or preventing action due to fear of falling. There can be a reduction in balance and strength due to a decrease in an activity which may make them dependent and long-term care. Fear of Fall is linked to increased falls, functional limitations, reduced quality of life and decreased Physical Activity levels. Although Fear of fall can result in additional care while performing any activity, likely avoiding accidents, the reduced activity can also contribute to deconditioning and decrease in muscle strength.(12)

**Rationale:**

Various studies have been carried out to find physical activity in Total Knee Replacement patients. But there is less evidence of fear of fall associated with Total Knee Replacement patients. Therefore, a need was felt to assess fear of fall in Total Knee Replacement patients along with the physical activity and to find out co-relation between physical activity and fear of fall in Total Knee replacement patients.

**Objectives:**

1. To assess physical activity in patients with Total Knee Replacement.
2. To Assess the fear of fall in patients with Total knee replacement
3. To find Correlation of physical activity with fear of falling in patients with Total knee Replacement.

**Methodology**

The study will be conducted in the OPD of Community Health Science in Ravi Nair Physiotherapy College, SawangiMeghe, Wardha. The Institutional ethics committee clearance will be obtained before the commencement of the study. Initially, the Patient will be thoroughly evaluated. After satisfying selection criteria the patient will be selected for the study. Informed consent will be obtained in order to participate in the study. The subjects undergone Total knee replacement (Bilateral /Unilateral) in Age group of 50-80 years will be included. Patients with Neurological disorder resulting in impaired balance and Patients with vestibular
disorders will be excluded. PASE Questionnaire will be used to assess physical activity in the elderly and the fall efficacy scale will be used to assess fear of falls in older population. Reading will be spread on a master sheet. Data will be analyzed statistically.

**Outcome measures:**

1. Fall Efficacy Scale to assess fear of fall.
2. PASE score to assess Physical activity.

**METHODS:**

**Study Design:** Observational Study

**Study Setting:** Physiotherapy OPD, Ravi Nair Physiotherapy College and AVBRH hospital Sawangi Meghe, Wardha

**PARTICIPANTS:**

**Inclusion criteria:**

1. Total knee replacement patient Bilateral / Unilateral
2. Age group 50-80 years
3. Both Genders

**Exclusion criteria:**

1. Patients with Neurological disorders resulting in impaired balance.
2. Patients with vestibular disorders.

**VARIABLES:**

1. Fear of fall
2. Physical activity

**DATA SOURCE/MEASUREMENT:**

For fear of fall- Falls efficacy scale score will be measured

For Physical activity- the score on the PASE scale will be measured

**Bias:**

Subjects not fulfilling the selection criteria will be excluded from the study to prevent bias.

**Study Size:** 100

**Statistical method:** Convenient Sampling Technique.

**Result:** Upon completion of the study results, statistical analysis will be estimated.

**Discussion**

The current study is carried out to find a correlation of fear of falls with Physical activity in Patient undergone total knee replacement various studies have shown that reduced physical activity and fear of falls is associated with Total Knee Replacement(2,5). Fear of fall may result in reduced physical activity and interchangeably if the physical activity is reduced after undergoing Total Knee Replacement may result in musculoskeletal changes causing decrease muscle strength and Range Of Motion affecting balance and inducing fear of falls. Thus, the current study aims to find out whether there is the correlation of fear of falls and physical activity in patients with Total Knee Replacement.

**Key Result:** Total knee replacement, Fear of fall in elderly, Physical activity in elderly, PASE Scale, Fall Efficacy Scale, FES, Elderly population, falls

**Limitation:** It might be difficult to convince the patient for being a part of this study.

**Generalisability:** Study not done yet.

**References**


Case Report

The Risks of Femoral Nailing in the Positioning of Hemilithotomy on Traction Table Getting A Contralateral Well-Legdrop-Foot

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Abstract

Introduction: Postoperative contra lateral morbidity following fracture fixation surgery is rare due to hemilithotomy placement on traction table. Following a typical orthopedic femoral nailing, we should note a case of unexplained typical peroneal nerve palsy formed on the contra lateral side, manifesting with drop foot.

Case report: After prolonged femoral nailing, a 32-year-old male suffered an uncommon common peroneal nerve palsy that manifested itself toward lateral drop foot. This iatrogenic and intermittent disorder was delineated to be position-related neuropraxia after neurophysiological analysis and review of applicable literature.

Conclusion: Place modification at intervals or complete avoidance of excessive hyperflexion of the knee is advised to prevent typical peroneal nerve morbidity against the lateral.

Keywords: foot drop, fracture of shaft of femur, common peroneal nerve palsy

Introduction

This study describes an extremely severe peroneal nerve palsy that formed following a severe orthopedic femoral nailing on the contra lateral leg, manifesting with drop foot (FIGURE 1). Fortunately, after a 4-month, cautious treatment, this functional deficiency was intermittent and fully recovered. So surgeons must be mindful of this possible morbidity when conducting surgery.

Case Presentation:

A 32-year-old male (body mass index of 30.2 kg/m²) sustained a comminuted, spiral fracture over his right femur in a motor vehicle accident. He was alert and focused on physical examination in the emergency department, with a strong contra lateral leg. Initial neurovascular examination on both legs were normal. The fracture was then treated using an intramedullary nailing with a closed reduction and stabilization. The patient underwent these surgical procedures under general anesthesia, and the unoperated leg was placed in a supine hemilithotomy position by a boot. It took four hours to achieve a suitable fixation, leading to technical difficulties in closely aligning fragments as well as in locking distal static screws. Fixation acceptable when the patient recovered consciousness he began complaining of extreme numbness and unable to move the unoperated leg.
His left leg was without swelling and local heat. However, at 24 hours after surgery, he already had a left foot drop showing complete loss of ankle dorsiflexion as well as impaired sensation under the knee, especially at the foot dorsum. The motor activity of the leg involved did not change during the remaining stay, while his sensory test progressed to a classic typical peroneal palsy distribution. The patient was fitted with an ankle-foot orthosis and was given physiotherapy with symptoms consistent with a severe peroneal nerve palsy. The velocity of the motor nerve conduction (NCV) indicated decreased amplitude in the common left peroneal nerve and needle electromyography (EMG) showed no motor unit action potential (MUAP) in the anterior left tibialis muscle, which was consistent with typical left peroneal neuropathy. He had daily outpatient follow-ups at our rehabilitation clinic after discharge, and was taught home exercises and given electrical stimulation. Both the motor and sensory functions of his left leg showed incremental progress. Two months after the incident he was almost able to remove his orthoses, and in the 4-month follow-up was observed a complete recovery of the unoperated leg without permanent sequelae.

**Clinical findings:**

Neurological findings included: foot manifesting complete loss of ankle dorsiflexion as well as impaired sensation below the knee, particularly at the foot dorsum there decreased pin-prick sensation and dragging of the left foot during gait study, with impaired dorsiflexion on heel strike.

<table>
<thead>
<tr>
<th>Table 1- Manual muscle testing (strength) assessment on 1 day of treatment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Knee</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ankle</td>
</tr>
<tr>
<td>Toe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table No : 2 Range of motion assessment on 1st day of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Hip flexion</td>
</tr>
<tr>
<td>Hip extension</td>
</tr>
<tr>
<td>Hip abduction</td>
</tr>
<tr>
<td>Hip adduction</td>
</tr>
<tr>
<td>Knee flexion</td>
</tr>
<tr>
<td>Knee extension</td>
</tr>
<tr>
<td>Ankle dorsiflexion</td>
</tr>
<tr>
<td>Ankle plantarflexion</td>
</tr>
</tbody>
</table>
**Diagnostic assessment:**

The velocity of the motor nerve conduction (NCV) indicated decreased amplitude in the common left peroneal nerve and needle electromyography (EMG) showed no motor unit action potential (MUAP) in the anterior left tibialis muscle, which was consistent with typical left peroneal neuropathy. Laboratory testing was done. Studies on nerve conduction and electromyography have confirmed the diagnosis of a severe peroneal neuropathy. Studies of nerve conduction showed potential amplitudes of a conductive block across the fibular head and below-normal compound motor activity. Slowing of the conduction was not observed. It was common to have the sensory and motor nerve responses in both legs. Electromyography of the right anterior tibialis muscle showed improvements in active and chronic denervation.

**Therapeutic intervention:**

- Intervention type in physiotherapy exercise
- self care
- ankle foot orthosis
- electrical muscle stimulation
- home program

**plan of care decide as per month:**

1 MONTH

- Electrical muscle stimulator: 30 contractions
  - sets × 3 times set
- Isometric dorsiflexion: 15 sec hold, repetition
  - 10 times (FIGURE 2(a))
  - Isometric planterflexion: 15 sec hold, repetition
  - 10 times (FIGURE 2(b))
- Wedge board standing
- Providing ankle foot orthosis: An orthotic ankle-foot is given that treats the steppageate by preventing drop in the foot during movement.

2 MONTH:

- Isometric dorsiflexion (active assisted movement) 15-20 sec hold, repetition - 15 times [FIGURE 2(a)]
- Isometric planterflexion: 15-20 sec hold, repetition 15 times [FIGURE 2(b)]
- Electrical muscle stimulator: 30 contractions × 3 times set
- Wedge board standing: 10 mins
- Toe to heel plantar flexion 10 sec hold for 10 min

3 MONTH:

- Electrical muscle stimulator: 30 contractions × 2 times
- Marble picked up exercise × 10 times
- Toe curl exercise × 7 to 8 times

4 MONTH:

- Electrical muscle stimulator × 30 contractions
- All foot movement will be actively performed
- Isometric dorsiflexion/plantarflexion
- Home program exercise

**Table 1- Manual muscle testing (strength) assessment on 1 day of treatment.**

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Pre-assessment</th>
<th>Post-assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Hip Extension</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Knee</td>
<td>4+</td>
<td>5</td>
</tr>
<tr>
<td>Knee Extension</td>
<td>4+</td>
<td>5</td>
</tr>
<tr>
<td>Ankle</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>Toe Extension</td>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>
**Table No 2: Range of motion assessment after rehabilitation**

<table>
<thead>
<tr>
<th></th>
<th>Active ROM</th>
<th>Passive ROM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip flexion</td>
<td>0-1150</td>
<td>0-1250</td>
</tr>
<tr>
<td>Hip extension</td>
<td>0-150</td>
<td>0-250</td>
</tr>
<tr>
<td>Hip abduction</td>
<td>0-350</td>
<td>0-450</td>
</tr>
<tr>
<td>Hip adduction</td>
<td>0-400</td>
<td>0-500</td>
</tr>
<tr>
<td>Knee flexion</td>
<td>0-1250</td>
<td>0-1350</td>
</tr>
<tr>
<td>Knee extension</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Ankle dorsiflexion</td>
<td>0-150</td>
<td>0-200</td>
</tr>
<tr>
<td>Ankle plantarflexion</td>
<td>0-350</td>
<td>0-450</td>
</tr>
</tbody>
</table>

**Outcome and follow up:**

After four months of therapy the dorsiflexion of his foot strengthened to grade 4/5.

---

**FIGURE 1: intramedullary nailing of femur shaft fracture**
Discussion

The common peroneal nerve is often involved in trauma in lower extremities, and typically occurs as a foot drop while damaged due to the paresis of its distributed ankle dorsiflexor, anterior tibialis, toe dorsiflexors, extensor digitorum brevis and extensor hallucis longus muscles. Postoperative drop foot on the contra lateral, uninjured leg is uncommon in literature and occurs only in case reports. Several causes contribute to common peroneal nerve neuropathy, including external compression (Plaster cast, brace or immobilization), direct trauma, traction injury, and entrapment in the fibular tunnel. The reinforcement of the flexed knee and calf significantly raises intramuscular pressure (direct compression theory) and reduces blood pressure in the ankle (vascular insufficiency theory). Another cause of common peroneal neuropathy is prolonged unhealthy position which induces a syndrome of nerve trapping presenting as a drop in the foot.

Conclusion

We note an rare case of contra-lateral, common peroneal nerve palsy that formed following femoral nailing. Physiotherapy plays a major role in the treatment of foot drop patients. The primary goal of care is to increase the range of motions. Only modalities of manual therapy and electrotherapy performed satisfactorily on patient.

Ethical Clearance: The institute ethics committee clearance is obtained

Conflict Of Interest: Nil.

Funding Support: None

References

5. O’Brien S, Gallagher N, Spence D, Bennett D, Dennison J, Beverland DE. Foot drop following


Correlation between Workload and Work Environment with Work Stress

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Abstract

Competition in the business world nowadays caused many pressures on workers and resulting in work stress. During 2001, the problem of stress had cost the organization up to $ 300 billion in terms of reimbursing maintenance costs, workers’ compensation, absenteeism and labor turnover. In Indonesia, especially Jakarta, around 1.33 million residents experience stress. This figure reaches 14% of the total population with severe stress levels of 7-10% and acute stress of 1-3%. It was also reported that Health care costs were almost 50% higher for treating workers who experienced high levels of stress in their work. Previous studies had found out that work stress was caused by several factors including work environment and workload. The purpose of this study was to analyze the correlation between workload and work environment with work stress on the Maintenance and Repair Division workers of PT. PAL Indonesia (Persero). This research design was a cross-sectional study. The population in this study were all technician workers on the Maintenance and Repair Division of PT. PAL Indonesia (Persero) as many as 51 workers. The sample was calculated using a simple random sampling technique so that a sample of 49 workers was obtained in the Production Department, Maintenance and Repair Division of PT. PAL Indonesia (Persero) as many as 51 workers. The sample was calculated using a simple random sampling technique so that a sample of 49 workers was obtained in the Production Department, Maintenance and Repair Division of PT. PAL Indonesia (Persero). The study was conducted in June 2019. Variables in this study were work climate, noise, lighting, physical workload, mental workload, and work stress. The data were analyzed by using the chi-square test and spearman test because there are differences in the scale of the data on the variables. The results are: work climate is not related to work stress (sig 0.715 > α), noise is not related to work stress (sig 0.380 > α), lighting is not related to work stress (0.322 > α), physical workload is related to work stress (sig 0.004 < α), mental workload has no relationship with work stress (sig 0.377 > α). The conclusion of this study is that only physical workload has a correlation with work stress.

Keywords: Workload, Work Environment, Work Stress

Introduction

Along with the development of the business world in the current development era, competition in the business world both at home and abroad is getting tougher and more complex. This forces companies to look for new innovations in order to increase production efficiency and productivity of their workers. To realize this, the company is challenged to be able to move all organizational factors to work optimally. One factor that plays a very important role in an organization is the human or worker factor. Workers have full control on every activity of the company in order to achieve its objectives.

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Therefore, workers are required to perform well in the interests of the company. The high demands of companies on workers will cause pressure in workers and can cause work stress. Work stress is a feeling of stress experienced by employees in carrying out work. In addition, stress is a response in adjusting that is influenced by differences in each individual and his psychological processes, as a result of environmental actions, situations or events that produce too much psychological and physical demands of a person\(^{(1)}\).

Symptoms of work stress according to Beehr & Newman are divided into three, namely 1) Psychological symptoms, which are characterized by anxiety, tension, confusion, irritability, mental fatigue, depression, ineffective communication, boredom. 2) Physiological symptoms, physiological changes are characterized by symptoms such as feeling tired / tired, exhausted, dizzy, digestive disorders, respiratory problems, high blood pressure, sleep disorders, physical fatigue, skin disorders, increased heart rate. 3) Symptoms of behavior such as absenteeism, decreased performance and productivity, decreased quality of interpersonal relationships with family and friends, anxiety.

In addition to the work environment, factors that also influence the occurrence of work stress are workloads. Human work consists of two characteristics that is, mental and physical in nature, then each has a level of loading which is different. Too much loading rate high energy consumption allows overload for physical workload and “overstress” for mental work, conversely intensity too low loading is possible boredom in physical work and burnout or “understress” on mental work. So, effort is needed for intensity levels optimum loading between the two limits the extreme was and of course different between each individual\(^{(7)}\). Workload is something that arises from the interaction between the demands of tasks and the work environment used as a workplace. Workload is divided into 2 namely physical workload and mental workload\(^{(8)}\). High work demands in the company will cause a large workload given to workers. Winarsunu states that there is a correlation between workload with three strains, namely psychological, physical, and behavioral strains\(^{(9)}\). On this basis, the purpose of this research is to find out the relationship between work environment and workload with work stress.

**Material and Method**

The approach used in this study was a quantitative research approach. Based on aspects of data collection, this study is a type of observational research because the researchers only observe objects without giving any treatment or intervention act. The design of this study was a cross-sectional study because the data collection was carried out in only one time period, that is when the research was conducted in the field.

Data collection in this study was using the questionnaire method and laboratorium measurement. The data collected were then analyzed on the correlation between two variables. Data presentation techniques used in this study include: editing, scoring, and tabulating. The data obtained will then be processed using data processing software. The results of the processed data were then analyzed descriptively and presented in the form of a frequency distribution table and percentage analysis, as well as an analysis of the correlation significance test between the two variables. The data were analyzed by using the chi-square test and spearman test because there were differences in the scale of the data on the variables. The significance level could be seen from the significance value. If it was less than 0.05, it showed that there was a correlation between the analyzed variables. Most of the styles are intuitive. However, we invite you to read carefully the brief description below.

This study was conducted in the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya on June 2019. The population used in this study was 51 technician workers of the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya. The sample was calculated using a simple random sampling technique so that a sample of 49 workers was obtained in the Production Department, Maintenance and Repair Division of PT. PAL Indonesia (Persero). The research variables analyzed in this study consisted of the dependent and independent variables. The independent variable in this study was the work environment (consisting of work climate, noise and lighting) and workload (consisting of physical workload and mental workload). Meanwhile, the dependent variables in this study was work stress.
Findings

The Frequency Distribution of Work Environment

In this study, work environment variables on the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya consisted of work climate, noise and lighting at work. Those three variables were divided into two categories based on the conditions felt by the workers, either the workers feel disturbed or not disturbed. Based on Table 1, the majority of workers (89.8%) did not feel disturbed by the work climate. The remaining 10.2% or 5 workers feel disturbed by the work climate in their workplace. Furthermore, as many as 36 people or 73.5% of workers did not feel disturbed by noise that occurs in the workplace. The rest (26.5%) were disturbed by the noise. For lighting variables, the majority of workers (77.6%) did not feel disturbed by the lighting in their workplace. While the remaining 22.4% were disturbed.

Table 1. The Frequency Distribution of Work Environment

<table>
<thead>
<tr>
<th>Work Environment</th>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disturbed</td>
<td>Not Disturbed</td>
</tr>
<tr>
<td>Work Climate</td>
<td>Frequency (%)</td>
<td>5 (10,2)</td>
</tr>
<tr>
<td>Noise</td>
<td>Frequency (%)</td>
<td>13 (26,5)</td>
</tr>
<tr>
<td>Lighting</td>
<td>Frequency (%)</td>
<td>11 (22,4)</td>
</tr>
</tbody>
</table>

The Frequency Distribution of Workload

The workload in this study was divided into two, namely physical workload and mental workload. Each variable was being grouped into three categories: low, medium and high. Based on Table 2, it can be seen that the majority of technical workers was having a ‘moderate’ physical workload (44.9%). Furthermore, as many as 19 workers (38.8%) had ‘low’ physical workloads and the remaining 16.3% had ‘high’ physical workloads.

Table 2. The Frequency Distribution of Workload

<table>
<thead>
<tr>
<th>Workload</th>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Physical Workload</td>
<td>Frequency (%)</td>
<td>19 (38,8)</td>
</tr>
<tr>
<td>Mental Workload</td>
<td>Frequency (%)</td>
<td>15 (30,6)</td>
</tr>
</tbody>
</table>

The Frequency Distribution of Work Stress

Work stress in this study was divided into 3 categories, namely low, moderate, and high. Based on Table 3, it is shown that the majority of technician workers on the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya were having a work stress in the ‘moderate’ category (53,1%) or as many as 26 persons. The rest, 17 persons (34,7%) were having work stress in the ‘low’ category and 6 persons (12,2%) were having work stress in the ‘high’ category.

Table 3. The Frequency Distribution of Work Stress

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>17</td>
<td>34,7</td>
</tr>
<tr>
<td>Moderate</td>
<td>26</td>
<td>53,1</td>
</tr>
<tr>
<td>High</td>
<td>6</td>
<td>12,2</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>100,0</td>
</tr>
</tbody>
</table>
The Relationship between Work Environment and Work Stress

Work environment variables in this study on the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya consisted of work climate, noise and lighting at work. Based on Table 4, it is shown that each of the variables from work environment had no significant correlation with work stress as the significance value was > 0.05. So, it was concluded that there was no significant correlation be-tween work environment and work stress on the technician workers of the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya. The OSH policy and the provision of PPE were correlated with individual commitment. Those two variables had a relationship in the ‘moderate’ category and a positive relationship with individual commitment. It was shown from the p-value in the OSH policy and the provision of PPE that was less than 0.05.

Table 4. The Relationship between Work Environment and Work Stress

<table>
<thead>
<tr>
<th>Work Environment</th>
<th>Work Stress</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work Climate</td>
<td>Pearson Chi-Square 0.671</td>
<td>Not Significant</td>
</tr>
<tr>
<td></td>
<td>Asymp. Sig. (2-sided) 0.715</td>
<td></td>
</tr>
<tr>
<td>Noise</td>
<td>Pearson Chi-Square 1.933</td>
<td>Not Significant</td>
</tr>
<tr>
<td></td>
<td>Asymp. Sig. (2-sided) 0.380</td>
<td></td>
</tr>
<tr>
<td>Lighting</td>
<td>Pearson Chi-Square 2.264</td>
<td>Not Significant</td>
</tr>
<tr>
<td></td>
<td>Asymp. Sig. (2-sided) 0.322</td>
<td></td>
</tr>
</tbody>
</table>

The Relationship between Workload and Work Stress

Workload variables in this study on the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya consisted of physical workload and mental workload. Based on Table 5, it is shown that physical workload had a significant correlation with work stress (significance value 0.004 < α). Further, mental workload had no significant correlation with work stress (significance value > 0.05). So, it was concluded that only physical workload had a significant correlation with work stress, as for mental workload had no significant correlation with work stress.

Table 5. The Relationship between Workload and Work Stress

<table>
<thead>
<tr>
<th>Workload</th>
<th>Correlation Coefficient</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>Spearman’s rho</td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.400</td>
<td>Significant</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Mental</td>
<td>Correlation Coefficient</td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.129</td>
<td>Not Significant</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.377</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Literature Review will show that the results of the research carried out in accordance with some of the results of other studies. The correlation result showed that there was no significant correlation between work environment and work stress. This is not in line with the research results of Angwen, which stated that there is a positive relationship between the physical work environment and work stress on PT. Citrabuana Electric Stage\(^6\). Therefore, it can be said if the physical work environment is comfortable then work stress too will be temporarily low if the physical work environment is uncomfortable, then his work stress will also be high. As for stress is an output of the interaction between individuals and the work environment, in which it can be a threat and stressors for both psychologically and physiologically of an individual\(^10\). This is in line with the research result which stated that both the physical work environment and non-physical work environment affects work stress on employees at PT PLN (Persero) Distribution of East Java Malang Service Area\(^5\). Physical work environment is all things that are around where someone works, which directly or indirectly can affect in carrying out his work, while non-physical work environment is all things that happen in the company related to work relations, both relationships with superiors and relationships with colleagues, as well as relationships with subordinates\(^11\). Companies are required to be able to make a good work environment. Because, in running the employee’s work will interact directly with a work environment that is in each part of the company. So, the work environment will be very influential on the stress that will be received by employees or can also be interpreted that work environment is one of the ways that can be done to be able to control or minimize the stress received by employees. If interaction with the environment can going well will reduce the rate stress, besides that a good work environment will reduce fatigue and burnout at work\(^5\). Another study stated that the importance of the work environment for performance because of a good and positive work environment as well empower can increase employee work productivity\(^12\). Further discussion of the variables in the work environment on work stress, the correlation result showed that there is no significant correlation between work climate and work stress. In the previous studies on the relationship between work climate and work stress, there was various results. Recent research results found that work climate has an influence on work stress. Furthermore, it is found that organizational climate had a negative effect on employee work stress. This indicates that the healthier the climate of an organization, the lower level of employee work stress. Conversely, the unhealthier a climate in the organization, the higher the level of employee work stress\(^13\). Another research result stated the opposite and along with this research’s result found that climate the organization has no effect on work stress\(^14\). The results of the study explain that awareness or dysfunctional feelings of employees as a result of conditions that are felt at work and an employee’s psychological and physiological reactions to uncomfortable or undesirable conditions are not affected by quality the internal environment of the organization which is relatively ongoing, experienced by members organization, influence their behavior and can be described in terms of a set characteristics or nature of an organization such as structure, responsibilities, standards, appreciation, support and commitment. Hereafter, the correlation result showed that there is no significant correlation between noise and work stress. The previous studies showed various results on the relationship between noise and work stress. Shept in stated that there was a positive correlation between noise and work stress of the electrical industry employees\(^9\). In line, Dawson also stated that there was a positive relation between the perception of noise in the work environment with work stress on bank employees\(^15\). A noisy environment is a disturbance that can affect comfort and health, especially those originating from operational activities of machine tools. Noise is a stressor that can cause physical, psychological, and human behavior changes\(^16\). In contrast, Febriana stated that there was no significant role between perception of noise on work stress on PT. Hasnur Riung Sinergi’s employees\(^9\). Further, the correlation result showed that there is no significant correlation between noise and work stress. In contrast, another research resulted in the influence of lighting workspace against work stress on research subject caused additional light intensity done helps reduce decrease in physical factors and factors psychological factors that affect work stress\(^17\). Employees who work on the premises those with good lighting will work better because they can work more optimally compared to lower lighting. Besides, that good
lighting also affects employee psychology where they will feel cared for and will create a positive perception in the mind the employee. Good physical and positive perception is what will cause employees to work with productive and achieve high performance\(^{(11)}\).

Further discussion of the variables correlated with work stress were physical work-load and mental workload. Every job, of course will put a burden on the workforce or humans both physically and burden mentally, from an ergonomic standpoint, every workload received by someone must be appropriate or balanced well against physical ability, cognitive abilities and human limitations who receive the burden. Physical workload is the workload received from jobs that require physical energy such as lifting, pushing, transporting, while for mental workload is the difference between demands between workloads of a task with the maximum capacity of someone in motivated condition\(^{(18)}\). The result of this study showed that there was a correlation between physical workload and work stress. This is in line with the research result which stated that there is a positive relationship between workload and work stress on PT Citrabuana Electric Stage employees\(^{(6)}\). Thus, it can be said that if the workload is high then the work stress will also be high. Conversely if the workload is low, the work stress will also be low. Furthermore, the result of this study showed that there was no significant correlation between mental workload and work stress. In contrast, there is a relationship between mental workload and work stress on labor CSSD In-stallation of Surabaya Hajj General Hospital. The relationship between mental work-load and stress have a strong relationship in the same direction which mean a higher mental workload experienced by workers, the higher the work stress level experienced by the workforce at CSSD Installation\(^{(19)}\). In line, there was also a relationship be-tween mental workload and work stress on educational workers such as teachers and lecturers in Batam\(^{(7)}\). Mental workload which is greater than the body’s ability can cause an uncomort feelings (early phase), tiredness (overstress), injury, accident, pain, illness and decreased productivity (final phase). Inconversely, if the workload is smaller than the body’s ability, it can cause understress, boredom, saturation, lethar-gy, less productivity and illness\(^{(20)}\).

**Conclusion**

Based on the results of this study, it can be concluded that the majority of technician workers on the Maintenance and Repair Division of PT. PAL Indonesia (Persero), Surabaya did not feel disturbed by the work environment in the workplace. The research results showed that most of the workers experienced the moderate level of both physical and mental workload. They also experienced a moderate level of work stress.

From the study results, we can conclude that there is no significant relationship between work environment and work stress. Further, there is no significant relationship between mental workload and work stress. The significant relationship is only found between physical workload and work stress.

**Conflict of Interest:** None.

**Source of Funding:** This study was sponsored by Universitas Airlangga.

**Ethical Clearance:** Ethical clearance was obtained from the Ethics Committee of the Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia.

**References**

7. Zetli S. Hubungan Beban Kerja Mental Dan Stres
The Role of Ultrasonography in The Diagnosis of Oral and Maxillofacial Disease

Ni Putu Mira Sumarta¹, David Buntoro Kamdjaja², Roberto Manahan Yantie Simandjuntak³
¹Researchers staff in Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya-Indonesia

Abstract

Objective: Ultrasonography, as a diagnostic tool, constitutes a non-invasive, cost-effective, readily-available and repeatable imaging technique. Ultrasonography has been used as a means of diagnosing various medical conditions for many years. However, in the field of maxillofacial surgery it represents a relatively new aid in the diagnosis of various diseases affecting the oral and maxillofacial regions. These include: infection, soft-tissue related diseases and vascular anomalies which can be detected using Doppler ultrasonography. This article presents four cases, in which ultrasonography was employed to confirm diagnoses and act as a guide to treatment.

Methods: Four cases of soft tissue swelling and enlargement were diagnosed with the aid of ultrasonography, namely: a submasseteric abscess, a nasolabial cyst, a dermoid cyst and a left buccal space abscess caused by a foreign body (i.e. a fish bone).

Result: In the case of a submasseteric abscess, ultrasonography was used in confirming the diagnosis and therapy, while determining the maximal point of the abscess. In the cases of both cysts, ultrasonography highlighted well-defined cystic lesions with internal echo showing fluid accumulation, while in the buccal space abscess, an ultrasonogram confirmed the exact location of the fish bone.

Conclusion: Ultrasonography is a quick, widely-available, relatively inexpensive, painless procedure which can be repeated as often as necessary without risk to the patient. Thus, ultrasonography is a valuable diagnostic aid to the oral and maxillofacial surgeon in achieving early and accurate diagnosis.

Keywords: Abscess, Cyst, Maxillofacial abnormalities, Ultrasonography.

Introduction

Ultrasonography (USG), as a diagnostic tool, is a non-invasive, cost-effective, readily-available and repeatable imaging technique. Although used as a diagnostic tool in the treatment of various medical conditions since 1940, in the field of maxillofacial surgery it represents a relatively new diagnostic aid.¹,² Medical ultrasound devices use ultrasound waves of 2-20 MHz. USG technique is based on the variable acoustic impedance produced at tissue interphases as sound waves reflected at various organ surfaces to produce images.³ The reflected sound beam produces diagnostic anatomic information relating to the size, shape and internal structure of normal tissues and also pathologic processes. The time interval between the ultrasound wave’s being emitted from the transducer and the registering of the reflected wave produces a measurement of the distance between the skin and the organ and also the location of the pathology. The resulting information from the reflected waves is digitalized and thousands of such measurements generate an ultrasound cross-sectional image which is then recorded on the monitor in order to enable its interpretation. Ultrasonography images comprise: hypoechoic (low reflection of sound waves) that appear black, isoechoic (intermediate reflection of sound waves) that appear heterogeneously grey, and hyperechoic (high reflection of sound waves) that appear white. Bone tissue, empty space and water are generally hypoechoic, while bone margin is hyperechoic and muscular tissue is isoechoic.⁴

USG is used as an aid in the diagnosis of various diseases in the oral and maxillofacial regions such as infection, soft-tissue related diseases such as those afflicting the salivary gland, lymphnode reactions, cysts and neoplasm. Vascular anomalies can also be detected using Doppler ultrasonography.¹,³,⁵ Recently, USG became more popular in dentomaxillofacial region because of increasing radiation dose concerns and
economic limitations.  

The purpose of this clinical study is to present four cases of soft tissue swelling where USG was used as an aid in confirming diagnosis and supporting surgical treatment.

**Material and Methods**

Clinical study was conducted through retrospective medical record study of four cases of soft tissue swelling and enlargement in patients of the Oral and Maxillofacial Department, Universitas Airlangga Dental Hospital where diagnosis was confirmed using USG (GE), as well as an aid in surgical therapy.

**Case 1**

A 24-year old female presented swelling of the left cheek and inability to open her mouth after having her lower left first molar extracted ten days prior to admission. Clinical examination findings included: patient looking unwell, presence of a diffuse, hard and painful swelling in the left masseter region and limited ability to open the mouth (i.e., less than 1 cm wide). No fluctuation was encountered, while intraoral examination confirmed no signs of post-extraction infection of the socket. Clinical diagnosis of a submasseteric space abscess was conducted and surgical drainage was planned. A USG examination completed for confirmation revealed a hypoechoic lesion in the submasseteric region with fluid echo intensity at a maximal point of 1.57 cm. The presence of a submasseteric abscess was confirmed by means of USG examination (figure 1).

![Figure 1. Ultrasonogram showed hypoechoic lesion (x sign) in submasseteric space abscess.](image-url)
An intraoral incision with Swann Morton surgical blade no.11 was performed to evacuate pus from the submasseteric space abscess, 1 cc pus mixed with blood being drained. On evaluation, pain was found to have diminished, mouth opening had widened and the swelling had gradually subsided.

Case 2

A 59-year-old male attended with painless enlargement of the left nasal base which had been developing during the ten years prior to admission. Clinical examination revealed soft tissue enlargement with defined borders in the left nasolabial region resulting in a narrowing of the left nasolabial sulcus when compared to the right side, measuring ± 2 cm in diameter with a soft consistency and painless on palpation. Intraoral examination revealed a flattened left upper anterior vestibulum due to enlargement with defined borders, measuring ± 2 cm in diameter, with a soft consistency and painless on palpation. Periapical radiograph examination was within normal limits (Figure 2A). Diagnosis of a nasolabial cyst was made and confirmed by means of USG which showed a thin walled cystic lesion with defined border and internal echo, measuring 2.49 x 1.87 x 2.39 cm in the left nasolabial fold (Figure 2B).

Extirpation of the cyst was performed through an intra oral approach, healing was effective and no recurrence was found upon evaluation.

Figure 2. Periapical radiograph showed normal appearance in nasolabial cyst (2A). Ultrasonogram showed cystic lesion in the left nasolabial fold (2B).

Case 3

A 25-year-old female had complained for the previous 12 years of a recurrent neck swelling that forced her tongue upwards and backwards, thereby impairing both her breathing and ability to swallow. She also complained of pain and fever, but was generally in good condition. The submental swelling was erythematous, well-defined, measured 4 cm in diameter and extended sublingually, pushing the tongue upwards and backwards. The consistency was firm and tender on palpation. Submandibular node enlargement was evident.

Clinical diagnosis of the infected dermoid cyst was conducted and, after aspiration made from the submental region for decompression revealed an extremely viscous, yellowish fluid, the patient was sent for USG examination. The USG revealed a well-defined heterogeneous echoic lesion, measuring 3.34 cm
in diameter, with mixed content and debris. There was also post-aspiration defect with adjacent fluid collection and no intralesion vascularization was found. The submandibular glands were enlarged and diagnosis of an infected dermoid cyst was confirmed (figure 3).

The treatment consisted of extirpation of the cyst through an extra oral approach under general anesthesia, healing was effective and no recurrence was found upon evaluation.

Figure 3. Ultrasonogram of Dermoid cyst showed a well defined heterogenous echoic lesion measuring 3.34 cm in diameter, with mixed content and debris. There was also post aspiration defect with adjacent fluid collection, and no intralesion vascularization found.

Case 4

A 68 year old male presented with swelling on his left buccal region that had been present with intermittent pain for one week prior to admission. The patient had suffered a fish bone puncture to the left buccal region upon eating three months previously. Clinical examination confirmed well-defined erythematous swelling in the left buccal region measuring 2 cm in diameter, with firm consistency and little pain on palpation. Well-defined intraoral swelling in left buccal mucosa measuring 2 cm in diameter, with a soft consistency and tender on palpation. An initial, provisional diagnosis was one of a left buccal space abscess caused by a foreign body (fish bone). This was subsequently confirmed by USG which revealed a foreign body (fish bone) in the left buccal region measuring 7 mm and 8.4 mm in depth from the skin surface. There was also adjacent fluid collection around the fish bone, edematous surrounding tissue and increased vascularization (figure 4).

Surgical drainage and exploration of the fish bone using an intraoral approach was performed. Upon
exploration, a 7 mm long fish bone was found and evacuated. The healing process was successful and no recurrence found.

![Ultrasonogram showing foreign body](image)

**Figure 4.** Ultrasonogram showed foreign body measuring 7 mm and 8.4 mm in depth from skin surface in the left buccal region (yellow arrow). There was also adjacent fluid collection around the fish bone, oedematous surrounding tissue, and increased vascularization in buccal space abscess caused by foreign body.

**Result**

All cases presented showed that USG 100% accuracy in diagnosis of soft tissue enlargement in oral and maxillofacial region, as well as guiding surgical drainage and approach.

**Discussion**

USG has been traditionally employed in the assessment of soft tissues in the abdomen and pelvis. Its role in oral and maxillofacial surgery is less widely recognized. Recently, a considerable body of literature and research reports about the reliable use of USG in diagnostic processes relating to oral and maxillofacial lesions. Research into the accuracy, sensitivity, specificity and predictive value of ultrasound as a means of diagnosis of cervico-facial soft tissue swelling conducted by Akinbami, et al. (2006) confirmed the reliability of ultrasonography in the diagnosis of pleomorphic adenoma as being 80% and 100% for adenocarcinoma and hemangioma. It was also 100% in the majority of cyst and salivary gland swellings. Ultrasonography was also 100% specific in the diagnosis of monomorphic adenoma and hemangioma. Research conducted by Chandak et al., (2011) to evaluate USG in the diagnosis of head and neck swelling, showed that this form of diagnosis provided a sensitivity and accuracy rate of 98.5% compared to that of clinical diagnosis at 85.7%.

All of the four cases reported in this paper showed congruence between clinical, ultrasonographic and histopathological diagnosis. This is consistent with the findings in the research reports referred to above.
In aiding therapy such as abscess drainage, ultrasonography can delineate the location and extent of abscess formation. USG is capable of measuring the distance from skin to oral mucosa, denoted a third dimension of the swelling and quantification of pus through its anechoic pattern and inflammatory zone. Ultrasonography can also be used intraoperatively to aid in the aspiration, incision and drainage of pus. As in the case of submaseteric abscesses and buccal space abscesses discussed in this report, the distance of the maximal point of the abscess, the location and distant of fish bone from the skin can be detected using ultrasonography.

**Conclusion**

Ultrasonography is a quick, widely available, relatively inexpensive, painless procedure which can be repeated as often as necessary without risk to the patient. Thus, ultrasonography is a valuable diagnostic aid to the oral and maxillofacial surgeons for early and accurate diagnosis, as well as in surgical treatment of oral and maxillofacial soft tissue enlargement.

**Ethical Clearance:** Nil.

**Sources of Funding:** Self-funded.

**Conflict of Interest:** There are no conflict of interest.

**References**


Association of Gamma-GT Serum and Nerve Conduction Velocity of Nervus Peroneus Motor Vehicles on Diabetic Polyneuropathy Patients

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Abstract

Background and Objectives: Diabetic Polyneuropathy is one of the common complications of diabetes mellitus (DM) and can lead to foot ulcers or amputation. The pathophysiology of Diabetic Polyneuropathy includes several factors such as metabolic, vascular, autoimmune, oxidative stress and neurohormonal growth-factor deficiency. The recent studies have suggested the use of serum gammaglutamyl transferase (GGT) as an early marker of oxidative stress. Therefore, we investigated whether serum GGT may be useful in predicting Diabetic Polyneuropathy.

Methods: The study was conducted in patients with diabetic polyneuropathy who meet the criteria for inclusion and exclusion in neurological outpatient clinic and EMG room departments of Neurology Dr.Soetomo General Hospital Surabaya consecutively started in June 2014 to April 2015.

Result: We obtained 20 study subjects (8 subjects with increased serum levels of gamma GT and 12 subjects with normal serum levels of gamma GT). There was statistically no significant between serum levels of gamma GT with motoric nerve conduction velocity (NCV) of peroneal nerve (p = 0.582 ; Odds ratio 0.600).

Conclusion: There was no relationship between elevated levels of serum gamma GT with decreased motor nerve conductivity velocity peroneal nerve in patients with diabetic polyneuropathy.

Keywords: Polyneuropathy, Gamma GT, Motoric, Nerve Conduction Velocity

Introduction

Neuropathy is a classic complication of diabetes. Distal polyneuropathy is the most frequently progressive diabetic neuropathy that progressively manifests slowly, symmetrically with glove and stocking patterns1. Neuropathy is most common in diabetics patients age over 50 years, rarely happen in age under the 30s and very rarely in children2.

Neuropathy is a major complication of diabetes that conduces in high morbidity rates. There is a strong association between hyperglycemia and progression of neuropathy reported in many studies. Oxidative stress plays an important role in the etiology and pathogenesis of diabetes. There is a change in the production of reactive oxygen species (ROS) in mitochondria and antioxidant defense systems of mitochondria3. Hyperglycemia, auto-oxidation of glycated proteins, increased production of reactive oxygen species (ROS), decreased antioxidant
defense, increased lipid peroxidation and membrane degeneration are the main causes of apoptosis or necrosis, which is common in diabetes 4.

The process of oxidative stress causes damage to cells and occurs in several types of cells in the nerves, including neurons (in axons and nerve terminals), glial cells, vascular endothelial cells. There are many changes that trigger the activation and recruitment of macrophages, which causes an inflammatory mechanism of stress and cell death. Until there is a difference from cellular stress happened that causes cell dysfunction or death and shows clinical manifestations as a neuropathy 5.

Early detection of diabetic neuropathy is very important in diabetes patients because prevention could reduce morbidity and mortality, but there is no gold standard to diagnose polyneuropathy. The San Antonio consensus recommends that a diagnosis of polyneuropathy should be measured in at least one of the five categories that measured by symptom scores, physical examination scores, quantitative sensory testing (QST), cardiovascular autonomic function (cAFT) and electrodiagnostic 6. Therefore, this study aims to determine the correlation of serum Gamma-GT levels and the nerve conduction velocity of Motor Peroneus Nerve in Diabetic Polyneuropathy Patients.

**Methods**

This study is cross-sectional to determine the association between serum Gamma-GT levels and motor peroneus nerve conduction velocity in type 2 of diabetes mellitus (DM) patients with polyneuropathy. This research was conducted between June 2014 to April 2015 in Nerves Unit of Dr. Soetomo General Hospital, Surabaya. The sample of the study was diabetic polyneuropathy patient who had outpatient treatment. The inclusion criteria were; patients suffering from diabetic neuropathy based on the Toronto criteria, <65 years old, were willing to participated in the study. As for the exclusion criteria were patients who have liver disorders, kidney disorders, heart problems, history of alcohol consumption, and obesity. The sampling technique of this study using consecutive sampling 7.

This research procedure begins when all the subjects included in the inclusion criteria and the responsible family of the patient are informed of the purpose, usefulness, and the risk of the study. At the end of the explanation, the subject or family was asked to read and ask questions about things that have not been understood. If they have understood and agreed, they were required to sign an approval letter 8. Subjects who have signed the letter of consent recorded their identity and characteristics in the form. The data collection of research subjects was conducted by author and residents in training, with the following steps: a careful history, physical examination and neurologic, sample selection for appropriate cases of inclusion and exclusion criteria, recording of eligible samples and all clinical data required 9.

All the data collected was processed statistically using SPSS 22.0 (SPSS. Inc. Chicago IL). Univariate data analysis was used to describe each variable, either independent or dependent variable from case group and control group with frequency distribution table. The bivariate analysis was used to determine whether there a relationship between two variables, or can also be used to determine whether there a difference between independent variables with dependent variables by using chi-square test 10.

For interpretation of results, this study uses the degree of significance (α) of 5%, with a note if p <0.05 then Ho is rejected, if p> 0.05 then Ho accepted 11. While to know the amount of risk factor, then used Odd ratio analysis (OR) with interpretation as follows; if OR = 1 means that the variable suspected as a risk factor has no effect on the occurrence of an effect, if OR> 1 and the range of confidence interval do not include number 1, then the exposure is a risk factor of effect, if OR <1 and the range of confidence interval do not include number 1, then the exposure under study can reduce the occurrence of effects (preventing factors) 12.

**Result**

In this research, there were 20 subjects of DM polyneuropathy patient that consisting of 11 subjects with normal Gamma GT levels, the rest was not normal and from 20 subjects it was obtained an increase of NCV (nerve conduction velocity) by 8 subjects while the rest decreased. The mean age was 51.85 years with standard deviation ± 4.793 using the Kolmogorov-Smirnov test 0.548 (p> 0.05) indicating that the normal distribution
(Table 1).

**Table 1. The relationship between age and and nerve conduction velocity (NCV) peroneus nerve motor**

<table>
<thead>
<tr>
<th></th>
<th>Nerve Conduction Velocity</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Averange Std.Dev</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>51.85 ± 4.793</td>
<td>0.548</td>
</tr>
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</table>

The overall subjects of the study were 20 subjects consisting of 4 men (20%) and 16 women (80%), wherein the group NCV (nerve conductivity velocity) decreased by 2 (50%) men and 6 people (37.5%) of women, whereas in the normal KHS group there were 2 (50%) male and 10 female (62.5%). The sex differences in each group were not statistically significant (p = 0.648) (Table 2)

**Table 2. The relationship between sex, DM duration, TG serum duration and nerve conduction velocity (NCV) peroneus nerve motor**

<table>
<thead>
<tr>
<th></th>
<th>Nerve Conduction Velocity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>Female</td>
<td>6 (37.5%)</td>
<td>10 (82.5%)</td>
</tr>
<tr>
<td>DM duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 Years</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>&lt;10 Years</td>
<td>5 (33.3%)</td>
<td>10 (66.7%)</td>
</tr>
<tr>
<td>TG serum duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5 (55.6%)</td>
<td>4 (44.4%)</td>
</tr>
<tr>
<td>Increased</td>
<td>3 (27.3%)</td>
<td>8 (72.7%)</td>
</tr>
</tbody>
</table>

In the subject group of DM polyneuropathy with decreased KHS has obtained 3 subjects (60%) who suffered DM >10 years and 5 subjects (33.3%) who suffer from DM <10 years, while patients with normal NCV got 2 subjects (40% ) who suffered DM 10 years and 10 subjects (66.7%) who suffered from DM <10 years old. The percentage difference in lengths suffering from DM was not statistically significant (p = 0.292) (Table.2). The subjects of polyneuropathy DM with NCV decreased by 5 people (55.6%) with serum triglyceride (TG) level and 3 (27.3%) patients whose serum TG levels increased while the patients with normal NCV were 4 (44.4 %) with normal serum TG levels and 8 people (72.7%) whose serum TG levels increased. The percentage difference in serum TG levels was not statistically significant (p = 0.199) (Table 2).

Serum Gamma-GT levels with NCV decreased by 5 subjects (45.5%) that considered as normal and 3 subjects (33.3%) with elevated levels. While, serum
Gamma-GT level with normal NCV was 6 subjects (54.4%) as normal and 6 subjects (66.7%) with increasing rate. The association of serum Gamma-GT levels with nerve conduction velocity in diabetic polyneuropathy patients was not statistically significant with p = 0.582. The value of Odds ratios obtained was 0.600, with a confidence interval range of 0.097 - 3.720 (CI 95%) (Table 3).

Table 3. The relationship between serum Gamma GT levels and nerve conduction velocity (NCV) peroneus nerve motor

<table>
<thead>
<tr>
<th>Gamma-GT serum level</th>
<th>Nerve Conduction Velocity</th>
<th>p</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decreased</td>
<td>Normal</td>
<td>0.582</td>
</tr>
<tr>
<td>Conduction Velocity</td>
<td></td>
<td></td>
<td>0.600 (CI : 95% ; 0.097 – 3.720)</td>
</tr>
<tr>
<td>Normal</td>
<td>5 (45.5%)</td>
<td>6 (45.5%)</td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>3 (33.3%)</td>
<td>6 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Based on the theory, on normal subjects, age and sex might be affected NCV. Older subjects had longer latency, smaller amplitude, and slower NCV than the younger subjects. While in female is have a higher amplitude, and difference in latency in each extremity than male. In one study 15% of people with type 2 diabetes had symptoms of neuropathy and almost 50% obtained NVC deceleration. Another case-control study with electrocardiographic-Nerve Conduction Velocity (EMG-NCV) test obtained significant differences in age and sex in diabetic neuropathy patients compared to normal control. However, this study found no significant association between age, and sex of NCV in diabetic polyneuropathy patients. This result was possible because of the research method used differently from previous studies.

The duration of DM patients also has an effect on the nerve conduction velocity, as in previous studies on the presence of complications of DM microvascular along with the length of DM associated with low nerve conductivity. Triglyceride levels also indirectly affect the nerve conduction velocity, hyperlipidemia is important in the development of diabetic polyneuropathy because elevated serum TG levels correlate with decreased myelin fiber density, independent of other variables such as age, DM length, DM control, and other variables.

In this study, there was no significant correlation between duration of type 2 diabetes and triglyceride levels with peroneus nerve conductive motor velocity in diabetic polyneuropathy patients. This was because there were differences in study design where this study using cross-sectional study that only shows the condition of the patient during the examination, while the process of poly polyneuropathy was a chronic process.

Theoretically, there is a close relationship between serum gamma-GT concentration and diabetic polyneuropathy in type 2 DM patients. This correlation also inversely proportional to nerve conductivity velocity. It was mentioned that the higher serum gamma-GT the lower the speed of nerve conduction. In a study that comparing the group of type 2 DM with the normal group showed a significant increases in serum gamma-GT (P <0.001) in all DM type 2 groups, whereas serum gamma-GT levels in diabetic polyneuropathy patients, were significantly increased compared to type 2 DM.
patients with velocity normal nerve conduction (65.7 Vs 40.6 IU/L, P = 0.002). The results of this study did not statistically found a significant relationship between serum Gamma-GT levels and nerve conduction velocity of motor peroneus. This may be due to different types of studies with previous studies, patients with controlled blood sugar, and may require a larger sample 17.

**Conclusion**

Based on the result of the research, it can be concluded that there was no correlation between serum gamma-GT level and nerve conduction velocity of motor peroneus in diabetic polyneuropathy patients.

**Ethical Clearance:** The research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**


In situ Molecular Hybridization of Kaposi’s Sarcoma Associated Virus (Human Herpes Virus 8) in Nasopharyngeal Carcinoma Tissues

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Abstract

Background: Viral, dietary and genetic factors are implicated in the etiology of nasopharyngeal cancer, a rare type of head and neck cancers. Unlike other viruses, HHV-8 encodes several human cytokines homologues and regulatory genes that play important roles in the viral pathogenesis.

Objective: To analyze the rates of HHV-8 infection in tissues obtained from a group of patients with nasopharyngeal carcinoma and inflammatory nasal polyps (INP).

Patients and Method: One hundred-thirty formalin-fixed, paraffin-embedded nasopharyngeal carcinoma and nasal inflammatory polyps tissues enrolled in this study; 65 nasopharyngeal tissue biopsies from nasopharyngeal carcinoma; 35 tissue biopsies from nasal inflammatory polyps and 30 nasopharyngeal tissues with unremarkable pathological changes, as apparently healthy tissue control. Detection of HHV-8 was done by chromogenic in situ hybridization (CISH) technique detection system.

Results: In nasopharyngeal carcinoma tissues, the HHV-8-DNA positive CISH reactions were detected in 23.1% while in nasal inflammatory polyps tissues HHV-8-positive CISH reactions were found in 8.6% of the examined tissues. The correlation between HHV-8 and NPC & INP was highly significant (P= 0.001).

Conclusion: Significant HHV-8 detection in nasopharyngeal carcinoma & inflammatory nasal polyp tissues could point for their possible role in either pathogenesis or carcinogenesis of both these lesions.

Keywords: HHV-8; Nasopharyngeal carcinoma; Inflammatory nasal polyps; CISH.

Introduction

Nasopharyngeal carcinoma (NPC) is a common aggressive and highly malignant tumor, arising from nasopharyngeal mucosa, and locally extending to the base of the skull, palate, nasal cavity or oropharynx. High incidence of cervical lymph nodes as well as distant metastases was reported (1). NPC are more vastly common seen in certain regions of East Asia and Africa than other regions. Viral, dietary as well as genetic factors were implicated in its etiology (2).

Epithelial head and neck malignancies demonstrate a relationship to oncogenic viruses including Human Papilloma virus, Epstein-Barr virus or Merkel cell polyoma virus, where oropharynx and respiratory tract were noticed as a common sites for the persistence and transmission of these oncogenic viruses (3,4).

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Kaposi’s sarcoma, a low-grade angioproliferative malignant neoplasm, is associated with human herpes virus – 8 infection, also known as the KS-associated herpes virus (KSHV)(5).

HHV-8 virions have morphological and structural features typical of other herpes viruses, including 3 proteins encoded by ORF 25, 26, and 62, however, a fourth protein, (encoded by ORF 65) lacks significant similarity to other herpetic viral counterparts(6). HHV-8 genome possesses approximately highly conserved 26 core genes, responsible for viral regulation, replication, and maturation (7). HHV-8 has at least 12 other human host gene homologs, not shared by other human herpes viruses, implicated in oncogenesis(8). In addition, a variety of gene products are encoded in HHV-8 for transformation, proliferation, cell signaling, antiapoptosis and angiogenesis, and immune modulation and immune evasion which may be involved in oncogenesis promotion and viral persistence(9).

Up to our best knowledge, the present study, represents the first in Iraq to analyze the rate of HHV-8 infection and to highlight a possible associative role of this virus in tissues obtained from a group of Iraqi patients with nasopharyngeal carcinoma (NPC) and inflammatory nasal polyps (INP).

**Material and Method:**

The detection of HHV-8 was performed on 4μm paraffin embedded tissue sections by chromogenic in situ hybridization (CISH) kit (purchased from ZytoVision GmbH. Fischkai, Bremerhaven. Germany), using digoxigenin-labeled oligo-nucleotides probe that targets HHV-8 DNA.

A probe complementary to a sequence of Human Herpes Virus -8 gene DNA was : Amount; 8.9 OD, 271 μg, 39.5 nmol; Length : 20-mer; GC content : 50 % Concentration (volume 1ml): 39.5 pmol/μl; Molecular weight : 6849 g/mol; Modification : 5’ Digoxigenin; Scale : 0.2 μmol; DMT : HPLC - Sequences of HHV8 Probe: (5’-ATG CAG CTA CAA CTT CGG AG-3’)A G C T; 6 5 5 4, respectively.

This study utilized SPSS program (version-21) for the statistical analysis, where Chi-Square test (χ2), Odd ratio and Spearman’s rho have been used to evaluate the differences between variables.

**Results**

I. Distribution of patients with nasopharyngeal lesions according to their Age: The nasopharyngeal cancer patients in this study were related to the age range from 18 -77 years and the mean age of those patients was (42.85 ± 14.89) years. Whereas the mean age of patients with inflammatory nasal polyps was (28.22 ± 17.76) years and their age ranged from 7 – 67 years and the mean age of apparently healthy individuals was (43.25 ± 6.99) years and their age ranged from 32- 64 years and as shown in table (1).

<table>
<thead>
<tr>
<th>Studied groups (Age/Year)</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Range</th>
<th>ANOVA test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy</td>
<td>30</td>
<td>43.25</td>
<td>6.99</td>
<td>1.44</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>individuals Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.006 sign. (P&lt;0.01)</td>
</tr>
<tr>
<td>Inflammatory nasal polyp(INP)</td>
<td>35</td>
<td>28.22</td>
<td>17.76</td>
<td>2.80</td>
<td>7</td>
<td>67</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma (NPC)</td>
<td>65</td>
<td>42.85</td>
<td>14.89</td>
<td>1.94</td>
<td>18</td>
<td>77</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
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</tbody>
</table>

II. Histological grading of Nasopharyngeal carcinoma (NPC): The grading of carcinoma group in the present study revealed that well differentiated (keratinizing) carcinomas constituting 10(15.4%) tissues of NPC group, while 2(3.1%) tissues of NPC have moderately differentiated grade. The poorly differentiated non- keratinizing grade was observed in 4(6.2%) tissues while undifferentiated carcinomas grade was observed in 49(75.4%) (Table 2). The statistical analysis of grading distribution of NPC revealed highly significant differences at (P<0.01).
Table (2): Nasopharyngeal carcinoma according to their grades.

<table>
<thead>
<tr>
<th>Grades</th>
<th>No.</th>
<th>%</th>
<th>Comparison of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>10</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>2</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>4</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>49</td>
<td>75.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

None of control tissues group presented positive signals for HHV-8-CISH test. However, in comparison to the percentage of HHV-8-DNA in healthy control group as well as in the group of INP, the differences between the percentages of HHV-8-DNA in tissues of patients with nasopharyngeal cancers and each of these groups are statistically very highly significant (P value = < 0.0001) and as revealed in (Table 3).

Table (3): Distribution of signal scores of HHV-8-DNA-CISH reactions.

<table>
<thead>
<tr>
<th>HHV-8 scores</th>
<th>A.H. Control</th>
<th>Inflammatory nasal polyp (INP)</th>
<th>Nasopharyngeal carcinoma (NPC)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>N</td>
<td>30</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>%</td>
<td>100%</td>
<td>91.4%</td>
<td>76.9%</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>N</td>
<td>0</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>%</td>
<td>0.00%</td>
<td>8.6%</td>
<td>23.1%</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>N</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>%</td>
<td>0.00%</td>
<td>5.7%</td>
<td>12.3%</td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>N</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>%</td>
<td>0.00%</td>
<td>2.9%</td>
<td>7.7%</td>
<td></td>
</tr>
<tr>
<td>+++</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>3.1%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>30</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Odds ratio</td>
<td></td>
<td>32.333</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

III. Human Herpes Virus -8-CISH expression in patients with nasopharyngeal & inflammatory nasal polyps:

Positive HHV-8 DNA-CISH signal scoring: Fifteen out of sixty five (23.1%) nasopharyngeal tissue biopsies with NPC showed positive CISH reactions for HHV-8-DNA (Figure 1). The Inflammatory nasal polyp (INP) tumors group revealed 8.6% positive signals which represented 3 out of 35 tissues in this group.

II. Signal intensity of HHV-8 - CISH testing: The signal intensities of HHV-8-CISH signal detection in NPC tissues group illustrated the strong signal intensity in (15.4%) whereas (6.2%) and (1.5%) have weak, and moderate intensity, respectively. In Inflammatory nasal polyp lesions, (5.7%) have moderate intensity; while...
(2.9%) have weak intensity. Statistically highly significant differences were recorded between studied groups at (P<0.01) as detailed in (Table 4).

**Table (4): Distribution of signal intensities of HHV-8 reactions.**

<table>
<thead>
<tr>
<th>HHV-8 intensities</th>
<th>A.H. Control</th>
<th>Inflammatory nasal polyp (INP)</th>
<th>Nasopharyngeal carcinoma (NPC)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>N</td>
<td>30</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100%</td>
<td>91.4%</td>
<td>76.9%</td>
</tr>
<tr>
<td>Positive</td>
<td>N</td>
<td>0</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.00%</td>
<td>8.6%</td>
<td>23.1%</td>
</tr>
<tr>
<td>weak</td>
<td>N</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.00%</td>
<td>2.9%</td>
<td>15.4%</td>
</tr>
<tr>
<td>Moderate</td>
<td>N</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.00%</td>
<td>5.7%</td>
<td>6.2%</td>
</tr>
<tr>
<td>strong</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>1.5 %</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>30</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Odds ratio</td>
<td></td>
<td>32.333</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

Figure (1): Microscopic appearance of HHV-8–CISH signals in nasopharyngeal carcinoma. Two patterns of blue–violet signals are observed at the site of complementary sequence in the cell nuclei of HHV-8-DNAA: A: Carcinoma with negative reaction (10X). B: Carcinoma with low score and moderate intensity reactions (10X).
IV. Correlations among the HHV-8 and the patient characteristics of nasopharyngeal carcinoma & Inflammatory nasal poly:

A strong positive relationship (with highly significant correlation) was found between HHV-8 and grade in NPC & INP ($r = 0.419, P = 0.006$). In addition, a strong positive relationship (with highly significant correlation) was found between HHV-8 and site of NPC & INP ($r = 0.483; p= 0.003$). However, there were no significant correlations among HHV-8 and age as well as gender (Table 5).

**Table 5. Spearman’s rho statistical testing of age, grade, HHV-8-CISH to evaluate the studied markers in in patients with NPC & INP.**

<table>
<thead>
<tr>
<th>Spearman’s rho</th>
<th>Age groups (years)</th>
<th>Grade</th>
<th>HHV-8</th>
<th>Site</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>r</td>
<td>-0.146</td>
<td></td>
<td></td>
<td>.125</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.352</td>
<td></td>
<td></td>
<td>-.034</td>
</tr>
<tr>
<td>Site</td>
<td>r</td>
<td>0.040</td>
<td>0.133</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.956</td>
<td>0.412</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV-8</td>
<td>r</td>
<td>0.172</td>
<td>0.419</td>
<td>0.483</td>
<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.376</td>
<td>0.006*</td>
<td></td>
<td>0.003*</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>.723</td>
<td></td>
<td>-.351</td>
</tr>
</tbody>
</table>

*Correlation is highly significant (P<0.01).

**Discussion**

In this study, the nasopharyngeal cancer patients have age range from 18 to 77 years and their mean age was $(42.85 \pm 14.89)$ years while the mean age of patients with inflammatory nasal polyps was $(28.22 \pm 17.76)$ years and their age ranged from 7 – 67 years (Table 1). The patients’ age in the present results coincides with the results of many other studies: Oga et al.\(^{(10)}\) and Parmar et al.,\(^{(11)}\) who found the mean age of Nigerian patients with head and neck cancers, including nasopharyngeal cancers, was 43.3 years. Several other studies are also in agreement with the current results, where nasopharyngeal cancers increased with the advancing age with a peak age of 41-60 years and decreased above 60 years\(^{(12,13)}\).

Also the current results could reflect that age is an important risk factor in nasopharyngeal tumorigenesis, where could be related by many risk factors that enhance appearance of malignant nasopharyngeal tumor in young age group in relation to the proceeding of age such as genetic predisposition, smoking and changes in life style (a highly caloric diet-rich in fat, refined carbohydrate, alcohol uptake)\(^{(14,15)}\).

Fifteen out of sixty five (23.1%) nasopharyngeal tissue biopsies with NPC showed positive CISH reactions for HHV-8-DNA (Figure 1). The Inflammatory nasal polyp (INP) tumors group revealed 8.6% positive signals which represented 3 out of 35 tissues in this group. None of control tissues group presented positive signals for HHV-8-CISH test.

Ablashi et al.\(^{(16)}\) found that out of 42 NPC patients, only two of these patients demonstrated antibodies to HHV-8. However, the complete lack of HHV-8 by PCR evaluation in\(^{(17)}\) study led them to hypothesize that HHV-8 is an unlikely etiologic candidate. HHV-8 infection was more prevalent in countries where classic and endemic KS occurred\(^{(18)}\).

HHV-8 encodes a viral homolog of interleukin 6, that induces increased motility, cell-cell and cell-substrate dyshesion and epithelial-to-mesenchymal transformation in breast cancer cells\(^{(19-21)}\).

The relationship between HHV-8 and NPC is based on the concept of identification of HHV-8 genome sequences in NPC tissues and immortalization of primary mammary epithelial cells by HHV-8. Although
the presence of HHV-8 is not enough for the tumorigenic transformation, yet it is expected to become an early events along cumulative changes over the years that become the starting step, similar to what happened in Kaposi’s carcinogenesis (22-25).

Acknowledgements: We ought to acknowledge the department of biology in college of science/university of Babylon and clinical communicable diseases research unit (Ccdru) college of medicine university of Baghdad for their permission to achieve the practical parts of this research.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

Funding: Self-funding

References
15. Li Y, Fu L, Wong AM, et al. Identification of


Smoke Exposure at Home to the Incidence of Pneumonia in Children Under 5 Years Old

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Abstract

Increased levels of pollutants in the room, apart from the penetration of outdoor pollutants, can also come from indoor pollutant sources such as cigarette smoke, smoke from using firewood, and the use of mosquito coils. Pneumonia is the ultimate overlooked child killer (major “forgotten killer of children”). In Klaten District, pneumonia cases in the last 3 years have increased, 1,068, 1,244, and 1,705. This study was to determine the relationship between the incidence of pneumonia and exposure to smoke at home in children under 5 years of age in Klaten Regency, Central Java Province. This type of research is observational analytic with case-control studies. The total sample of 278 (139 cases and 139 controls) children under 5 years of age resided in Klaten Regency and were recorded as pneumonia cases in the public health care register in 2012 that met the inclusion and exclusion criteria. The research instrument consisted of a structured questionnaire. The results of data analysis showed that the relationship with the incidence of pneumonia in children under five was the variable exposure to cigarette smoke (OR=2,053; \( p=0.017 \) and 95% CI=1,137-3,705) and exposure to kitchen smoke (OR=2,664; \( p=0.003 \) and 95% CI=1,4002-5,0680). Factors associated with the incidence of pneumonia in children under 5 years of age in Klaten Regency, Central Java Province are exposure to cigarette smoke and exposure to kitchen smoke.

Keywords: Risk factors, pneumonia, toddler

Introduction

The goal of health development that has been listed in the National Health System is an effort to implement health by the Indonesian nation to obtain the ability to live a healthy life for every community to achieve an optimal health degree, which is said to indicate that the increase in the degree of public health is influenced by several factors, namely the environment, health services, actions and congenital (congenital)¹.

Environmental conditions can affect public health conditions. Many aspects of human well-being are influenced by the environment, and many diseases can be initiated, supported, sustained, or stimulated by environmental factors. This means that the interaction between humans and their environment is an important component of health because humans need the carrying capacity of environmental elements for their survival, for example, air, water, food, clothing, and all human needs must be taken from their environment. Human health can only be affected by environmental conditions if the human is exposed to environmental factors at a level that cannot be tolerated²,³.

The house plays a very important role in human life, where the values of a family take place, it becomes a human space to express how to live, communicate, interact with the people closest to them. The housing and settlement problems in Indonesia are rooted in the shift
in population concentration from rural to urban areas. The growth of the urban population in Indonesia is quite high, around 4% per year, higher than the national growth, and tends to continue to increase\(^4\,^5\).

Indoor air pollution can be very dangerous because the source is in direct proximity to humans. In developing countries, an important problem of indoor air pollution is pollution in the house due to cooking or burning wood for heating without adequate chimneys. Other pollutants that hurt health are O\(_3\), ionizing radiation, and cigarette smoke. WHO estimates that every year there are about three million cases due to indoor air pollution and 0.2 million due to outdoor pollution\(^6\).

Based on research from the American College of Allergies, about 50% of diseases are caused by indoor air pollution. The United States Environmental Protection Agency (US EPA) states that indoor air pollution is two to ten times more dangerous than outdoor air. Scientific America reports that a baby crawling on the floor inhales carpet dust, mold, mildew, mites, etc. the equivalent of smoking four cigarettes a day. More than 90% of people spend their time indoors so indoor air pollution has more dangerous health impacts than outdoor air pollution\(^7\).

Increased levels of pollutants in the room, apart from the penetration of outdoor pollutants, can also come from indoor pollutant sources such as cigarette smoke, smoke from using firewood, and the use of mosquito coils. Pneumonia is one of the causes of high morbidity and mortality in children under five years of age (toddlers) in developing countries. Nineteen percent of deaths of children under five are caused by pneumonia. Three-quarters of pneumonia cases in the world are found in 15 countries and Indonesia is in the sixth rank\(^5\,^8\).

According to WHO and UNICEF, pneumonia is the major “forgotten killer of children”. Pneumonia is a higher cause of death when compared to the total deaths due to AIDS, malaria, and measles. Every year, more than 2 million children die from pneumonia, meaning 1 in 5 children under five dies in the world. Pneumonia is the most common cause of death, especially in countries with high mortality rates. Almost all deaths from pneumonia (99.9%), occur in developing and less developed countries (least developed)\(^9\,^10\).

Indonesia is one of the 15 countries and occupies the 6th place with 6 million cases. The Household Health Survey (SKRT) from the Ministry of Health in 1992, 1995, and 2001 showed that pneumonia had a major contribution to infant and child mortality. Whereas in the basic health research (Riskesdas) in 2007, pneumonia was in 2nd place as the cause of death for infants and toddlers after diarrhea and was in 3rd place as the cause of death in neonates\(^11\).

**Material and Method**

This type of research is an observational analytic study with case-control study design. The population in this study were all children under 5 years of age suffering from pneumonia in 3 sub-districts in rural areas and 2 sub-districts in urban areas and visited each Public health center.

The research locations were in 5 sub-districts, namely Pedan (Pedan Public health center), Tulung (Tulung and Majegan Public health center), gantiwarno (Switchwarno Public health center), Klaten Selatan (Klaten Selatan Public health center), and Kalikotes (Kalikotes Public health center). The population in this study were 139 cases and 139 controls. Data analysis of the research results was carried out in 3 stages, a namely univariate analysis which aims to describe the phenomena found, both in the form of risk factors and effects or results, bivariate analysis to determine the relationship of research variables to the incidence of pneumonia used the Mc Nemar test and multivariate analysis was carried out. with the conditional logistic regression multiple tests to find evidence that the independent variables are related to the dependent variable which is measured together.
Findings

Table 1. Description of Respondents According to Smoke Exposure at Home in Klaten Regency

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case</th>
<th>Control</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Cigarette smoke exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>84</td>
<td>60,4</td>
<td>62</td>
<td>44,6</td>
</tr>
<tr>
<td>No</td>
<td>55</td>
<td>39,6</td>
<td>77</td>
<td>55,4</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>100</td>
<td>139</td>
<td>100</td>
</tr>
<tr>
<td>Mosquito coils exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>83</td>
<td>59,7</td>
<td>69</td>
<td>49,6</td>
</tr>
<tr>
<td>No</td>
<td>56</td>
<td>40,3</td>
<td>70</td>
<td>50,3</td>
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<tr>
<td>Total</td>
<td>139</td>
<td>100</td>
<td>139</td>
<td>100</td>
</tr>
<tr>
<td>Kitchen smoke exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td>35,3</td>
<td>24</td>
<td>17,3</td>
</tr>
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<td>No</td>
<td>90</td>
<td>64,7</td>
<td>115</td>
<td>82,7</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>100</td>
<td>139</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Relationship Between The Incidence of Pneumonia and Smoke Exposure at Home in Children Under 5 Years Old in Klaten District

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>Total</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td></td>
<td>FR (+)</td>
<td>FR (-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoke exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (FR +)</td>
<td>45</td>
<td>39</td>
<td>84</td>
<td>2,3</td>
<td>1,267-4,325</td>
</tr>
<tr>
<td>No (FR -)</td>
<td>17</td>
<td>38</td>
<td>55</td>
<td>1,6</td>
<td>0,931-2,836</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>77</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosquito coils exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (FR +)</td>
<td>46</td>
<td>37</td>
<td>83</td>
<td>1,6</td>
<td>0,931-2,836</td>
</tr>
<tr>
<td>No (FR -)</td>
<td>23</td>
<td>33</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>70</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitchen smoke exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (FR +)</td>
<td>11</td>
<td>38</td>
<td>49</td>
<td>2,9</td>
<td>1,523-5,980</td>
</tr>
<tr>
<td>No (FR -)</td>
<td>13</td>
<td>77</td>
<td>90</td>
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</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>115</td>
<td>139</td>
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</table>
Table 3. Multivariate Analysis (Multiple Conditional Logistic Regression) Relationship Between Pneumonia Incidence and Smoke Exposure at Home in Children Under 5 Years Old in Klaten Regency

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>SE</th>
<th>Z</th>
<th>p-value</th>
<th>95% CI</th>
<th>Pseudo R²</th>
<th>LR</th>
<th>Prob&gt;Chi²</th>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoke exposure</td>
<td>2.04</td>
<td>0.617</td>
<td>2.35</td>
<td>0.019</td>
<td>1.127-3.689</td>
<td>0.1042</td>
<td>20.08</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mosquito coils exposure</td>
<td>1.372</td>
<td>0.39</td>
<td>1.11</td>
<td>0.266</td>
<td>0.786-2.394</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Kitchen smoke exposure</td>
<td>2.51</td>
<td>0.84</td>
<td>2.77</td>
<td>0.006</td>
<td>1.308-4.821</td>
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<td></td>
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<tr>
<td>Log-likelihood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-86,304995</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoke exposure</td>
<td>2.053</td>
<td>0.619</td>
<td>2.39</td>
<td>0.017</td>
<td>1.137-3.705</td>
<td>0.0977</td>
<td>18.84</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kitchen smoke exposure</td>
<td>2.664</td>
<td>0.87</td>
<td>2.99</td>
<td>0.003</td>
<td>1.400-5.068</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Log-likelihood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-86,929877</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Cigarette Smoke Exposure

Cigarette smoke is categorized as the most dominant cause of indoor air pollution. In a closed room, smoke collects with a concentration that varies according to the number of smokers in the room, the type of cigarette, the number of cigarettes smoked, and the characteristics of the room such as size, ventilation, temperature, and humidity. Smoking is not only dangerous for the health of the smoker but also dangerous for those around him who are accidentally exposed to the smoke (passive smoking), especially children who still have low immunity. Besides being carcinogenic, cigarette smoke can also cause airway irritation by sulfur dioxide, ammonia, and formaldehyde\(^{12,13}\).

Based on research in Klaten Regency, the results of the bivariate analysis showed that there was a significant biological and statistical relationship between cigarette smoke exposure and the incidence of pneumonia with \( p = 0.0046 \) at \( \alpha = 5\% \) (0.05); \( OR = 2 \); and \( 95\% \) CI = 1.267-4.325, so that continued with multivariate analysis obtained \( p\)-value = 0.017; \( OR = 2.053 \); and \( 95\% \) CI = 1.137-3.705, which means that there is a significant relationship between exposure to cigarette smoke and the incidence of pneumonia. The magnitude of the risk can be seen from the \( OR = 2.053 \) value, which means that children exposed to cigarette smoke will have an increased risk of developing pneumonia by 2.053 times greater than children who are not exposed to cigarette smoke.

Mosquito Coils Exposure

Most of the Indonesian people still use mosquito coils to repel mosquitoes because the price of mosquito coils is relatively cheaper. Mosquito repellent is very dangerous for our body, especially mosquito coils. Mosquito repellent includes drugs that contain toxic chemicals and are very harmful to the body when the
smoke is inhaled. Mosquito repellent is dangerous for humans because of the active ingredients belonging to the organophosphate group such as Dichlorovynil dimethyl phosphate (DDVP), Proxopur (Carbamate), and Diethyltoluamide, which are a type of insecticide that kill insects that are carcinogenic14,15.

The toxic content of mosquito repellents depends on the concentration of the poison and the amount of use and the effect on the body depends on the type, amount, age of use, and the ingredients of the mixture. Children are vulnerable to mosquito repellents because their organs are not yet perfect and their immune systems are not yet good. The active ingredients of the mosquito repellent will enter the body through the breath and the skin and then circulate in the blood and then spread to the body’s cells such as respiration, brain (through the central nervous system), and others. The greatest effect will be experienced by sensitive organs. Because, mosquito repellent is more about inhaled, so what is usually affected is breathing. Meanwhile, the side effects on the skin depend on the sensitivity of the skin. Disturbances in human organs will occur if the use of insect repellent is not controlled or the dosage is excessive. People who have allergies will react more quickly. The most common allergies usually affect the airways, causing a cough16,17.

The human airway is equipped with an epithelium or airway lining which has cilia like vibrating hairs that function to expel something. Cilia will react to secretions (mucus fluid) and foreign objects in the airway that will be expelled upward, however, the chemicals in mosquito repellents consist of active, irritating substances so that the epithelial cells and cilia are more easily damaged. If the epithelium and cilia are damaged, foreign objects cannot be removed. Besides, the cells under the epithelium will also be affected as a result, mucus will be released. Furthermore, the airway becomes contracted because the nerves are disturbed, resulting in coughing and difficulty breathing16,18.

Based on research in Klaten Regency, the results of the bivariate analysis showed that there was no significant biological or statistical relationship between exposure to mosquito coil smoke and the incidence of pneumonia with p = 0.0925 at α = 5% (0.05); OR = 1.6; and 95% CI = 0.931-2.836 because the p-value <0.25, then continued with multivariate analysis, the results obtained were p = 0.266 OR = 1.37 and 95% CI = 0.786-2.394 which means that there is no significant relationship between exposures. mosquito coils with incidence of pneumonia. The magnitude of the risk can be seen from the OR = 1.37, which means that children exposed to mosquito coil smoke will increase the risk of developing pneumonia by 1.37 times greater than children who are not exposed to mosquito coil smoke.

**Kitchen Smoke Exposure**

Based on research in Klaten Regency, the results of the bivariate analysis showed that there was a significant biological and statistical relationship between exposure to kitchen smoke and the incidence of pneumonia with a value of p = 0.0006 at α = 5% (0.05); OR = 2.9; and 95% CI = 1,523-5,980, so that continued with multivariate analysis obtained p-value = 0.003, OR = 2,664 and 95% CI = 1,400-5,068, which means that there is a significant relationship between exposure to kitchen smoke and the incidence of pneumonia. The magnitude of the risk can be seen from the OR = 2,664, which means that children who are exposed to kitchen smoke will have an increased risk of developing pneumonia by 2.664 times greater than children who are not exposed to kitchen smoke. This study was different from Salam (2006), which showed that there was no significant relationship between exposure to kitchen smoke and the incidence of pneumonia with p = 0.157; OR = 3.328, and 95% CI = 0.630-17.596.

Air pollution from burning traditional kitchen biomass (wood, charcoal, straw, former harvest) is a public health risk. Smoke pollutants produced from combustion are very dangerous to health and will cause various kinds of diseases, especially if the ventilation does not meet health requirements. Diseases that can be caused include ARI, asthma, lung cancer, cataracts, and tuberculosis. The smoke and soot when inhaled enter the respiratory tract to the lining of the lungs (mesothelium) so that the respiratory system will shrink (spasm), as a result, the elasticity of the lungs will decrease and the vascular pressure will increase. Biomass stoves produce pollutants, including carbon monoxide. Carbon monoxide is poison gas. The concentration of CO in the kitchen using biomass can reach 100 ppm per hour, far above the threshold set by the World Health Organization...
Conclusion

Based on the results and discussion of research, the relationship between the incidence of pneumonia and exposure to smoke in the home in children under 5 years of age in Klaten Regency can be concluded as follows: The research variable that was not statistically significant for the incidence of pneumonia in Klaten was exposure to mosquito coil smoke.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of Ibn Khaldun University.

All subjects were fully informed about the procedures and objectives of this study each subject before the study signed an informed consent form.

References


Factors Affecting Romantic Relationship Satisfaction of University Students

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Abstract

Background/Objectives: The purpose of this study was to identify the factors affecting romantic relationship satisfaction of university students.

Methods/Statistical analysis: This study was conducted with 187 students from four universities in cities and provinces nationwide. Data were collected from September to October 2019 using a self-administered questionnaire. Collected data were analyzed through the stepwise regression analysis using the SPSS statistics program.

Findings: The final regression model showed that self-esteem, anxiety attachment, and avoidance attachment were significant predictors related to romantic relationship satisfaction in university students and explained 34.7% of the variance in romantic relationship satisfaction.

Improvements/Applications: The university student period is a time to form an adult attachment, thereby preparing for a happy marriage in the future by increasing the romantic relationship satisfaction. Therefore, it is necessary to develop and apply the educational programs that can increase self-esteem and positive heterosexual relationship for university students.

Keywords: University students, sexual attachment, sexual attitude, self-esteem, romantic relationship satisfaction

Introduction

The time in university is a transitional stage from adolescence to adulthood, and it can be seen in the three main area of identity exploration: love, work, and worldviews which is the late period of adolescence and early period adulthood. In Korea, many young people begin to date only after entering university. A satisfactory experience through the romantic relationship with a partner enhances an individual’s psychological health and level of happiness, allows them to feel positive emotions, and influences the development of positive self-concept. Romantic relationship satisfaction refers to the subjective evaluation, attitude, and positive feelings about relationships, and attachment is one of key factors related to relationship satisfaction.

Adult attachment is the result of a process in which the attachment target shifts from the primary caregiver to friends and lovers as an individual enters into adulthood. For university students in the emerging adulthood, who are in the process of forming adult attachment, the experience of healthy romantic relationship is important in itself. In particular, individuals with stable attachment in relationships with partners have displayed positive characteristics such as confidence, happiness, and constructive approaches toward relationship conflicts, while individuals with high levels of attachment anxiety and avoidance showed negative characteristics such as discomfort, jealousy, etc., in their relationships. Therefore, in order for unmarried men and women to
maintain a satisfactory relationship with the opposite sex, the sexual aspect must be considered, and for this, an individual’s attitude toward sex is important.

Sexual attitude is one’s own cognitive perspective on an individual’s sexual aspect, is formed through sexual behaviors, and enables individuals to determine current and future sexual behavior based on it\[9\]. Positive sexual attitudes not only affect personality maturity, but also premarital and post marital sex life  

Self-esteem, meanwhile, is a key factor in determining an individual’s behavior and adaptation, and it is essential for the healthy sexual function in romantic relationships\[11\]. Specifically, Lee’s study\[12\], which finds that teenagers with low self-esteem are more exposed to unwanted sex, suggests that self-esteem is related to satisfaction in relationships. Prior studies in Korea frequently show that sexual attitude affects satisfaction in relationships, but the direction of the influence is different. Although the results are inconsistent, it is clear that sexual attitude is correlated with relationship satisfaction\[13\]. In addition, there are studies on adult attachment and satisfaction with romantic relationship, but there is a only of handful of research on the mechanism. Moreover, there is no research on an integrated approach to the factors affecting the satisfaction of relationships among university students. Therefore, this study aims to provide the necessary basic data to establish healthy romantic relationships and appropriate sexual attitudes of university students by identifying the determinants in relationship satisfaction of university students using variables of various aspects.

**Method**

**Subjects**

The subjects of this study were students of universities located in four cities and provinces in Korea and were convenient-sampled to male and female university students with dating experience. Using G*Power 3.12, the sample size of the study was set to be 160, where the median size effect is 0.15, a significance level is 0.05, and the number of predictor variables is 8, to secure 95% of the statistical power for regression analysis. Therefore, in this study, a total of 187 questionnaires were included in the final analysis after distributing the questionnaire to 192 people considering the dropout rate of 20%.

**Tools**

**Adult attachment**

The Experience in Close Relationship (ECRS), developed by Brennan et al.\[14\], was used. Adult attachment measurement tools consist of two areas: 18 questions for avoidance attachment and 18 questions for anxiety attachment. The higher the score on a 5-point scale of 36 questions, the higher the avoidance attachment and anxiety attachment. In this study, Cronbach’s $\alpha$ were found to be 0.74 (avoidance attachment), 0.87 (anxiety attachment), and 0.83 overall.

**Sexual attitude**

The sexual attitude measurement tool developed by Woo\[10\] was used. The tool was comprised of 35 questions with a 5-point scale. A higher score means a more open sex attitude, and a lower score means a more conservative sex attitude. Cronbach’s $\alpha$ in this study was found to be 0.74.

**Self-esteem**

The self-esteem measurement tool developed by Rosenberg\[15\] was used. The self-esteem measurement tool consists of 10 questions with a 5-point scale. A higher score means a higher degree of self-esteem. Cronbach’s $\alpha$ in this study was found to be 0.82.

**Romantic relationship satisfaction**

The romantic relationship satisfaction measurement tool developed by Lee\[16\] was used. The tool consists of 41 questions with a 5-point scale. A higher score means higher romantic relationship satisfaction. Cronbach’s $\alpha$ in this study was found to be 0.92.

**Data collection**

For the data collection, universities located in four different regions across the country from September 9 to October 31, 2019 were randomly selected. Through a research assistant at each local university, the purpose and method of the study were explained to the study subjects, and written consents from the subjects who exhibited voluntary participation were received. The research assistants explained that the collected data would not be disclosed or used for any purpose other than research, that the subject’s personal information would
be kept confidential and guarantee anonymity, and that the subject could stop participating in the research at any time if desired. The data were collected using structured questionnaires and it took 10~15 minutes to complete the questionnaire.

**Ethical considerations**

This study was conducted after receiving research approval from the Institutional Review Board (IRB) of University C to project the subjects in prior to conducting the study (IRB No: CKU-19-01-0207)

**Data analysis method**

The collected data were processed by computer statistics using SPSS/WIN 22.0 program. Descriptive statistics for the general characteristics and variables of the subjects were obtained. The difference in Romantic relationship satisfaction level was determined according to general characteristics t-test, ANOVA, and post-test Scheffé test. The correlation between the Romantic relationship satisfaction and the variables was analyzed by Pearson’s correlation coefficient. In addition, in order to identify factors affecting the Romantic relationship satisfaction, it was analyzed by stepwise multiple regression after multicollinearity diagnosis.

**Result**

**General characteristics of subjects**

Mean age of the subjects was 21.03(±2.06) years old and 20 to 29 years old group was the most among these with 134 persons (71.7%). The distributions of the gender were 95 male (50.8%), 92 female (49.2%). There were 1st 55(29.4%), 2nd 41(21.9%), 3rd 39(20.9%) and 4th 52(27.8%) in the Grade. Residence was living alone 82(43.9%), dormitory 72(38.5%) (Table 1).

**Table 1. General Characteristics and Difference in Degree of Romantic Relationship Satisfaction according to General Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Categories</th>
<th>n(%nt)</th>
<th>M±SD</th>
<th>Romantic relationship satisfaction</th>
<th>M±SD</th>
<th>t/F</th>
<th>p</th>
<th>Scheffé</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>Teens (10’s)</td>
<td>53(28.3)</td>
<td>2.60±0.52</td>
<td>0.09</td>
<td>.929</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Twenties (20’s)</td>
<td>134(71.7)</td>
<td>2.59±0.52</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.03±2.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Male</td>
<td>95(50.8)</td>
<td>2.53±0.54</td>
<td>-1.73</td>
<td>.085</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>92(49.2)</td>
<td>2.66±0.50</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td>1st</td>
<td>55(29.4)</td>
<td>2.56±0.49</td>
<td>2.42</td>
<td>.067</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>41(21.9)</td>
<td>2.76±0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>39(20.9)</td>
<td>2.62±0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>52(27.8)</td>
<td>2.48±0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td>Home</td>
<td>31(16.6)</td>
<td>2.60±0.47</td>
<td>0.38</td>
<td>.767</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dormitory</td>
<td>72(38.5)</td>
<td>2.64±0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Living alone</td>
<td>82(43.9)</td>
<td>2.56±0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Etc.</td>
<td>2 (1.1)</td>
<td>2.44±1.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Descriptive statistics of the study variables

Attachment averaged 2.78 (±0.58). In addition, the average sexual attitude was 3.04 (±0.36), and the self-esteem was 3.66 (±0.62) (Table 2).

Table 2. Descriptive Statistics of the Study Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avoidance attachment†</td>
<td>2.73</td>
<td>0.44</td>
<td>1.33</td>
<td>3.72</td>
</tr>
<tr>
<td>Anxiety attachment†</td>
<td>2.78</td>
<td>0.58</td>
<td>1.28</td>
<td>4.67</td>
</tr>
<tr>
<td>Sexual attitude</td>
<td>3.04</td>
<td>0.36</td>
<td>1.46</td>
<td>4.43</td>
</tr>
<tr>
<td>Self-esteem</td>
<td>3.66</td>
<td>0.62</td>
<td>1.30</td>
<td>4.90</td>
</tr>
<tr>
<td>Romantic relationship satisfaction</td>
<td>2.59</td>
<td>0.52</td>
<td>1.27</td>
<td>4.24</td>
</tr>
</tbody>
</table>

Difference in degree of romantic relationship satisfaction according to general characteristics

The degree of romantic relationship satisfaction was non-significantly different according to general characteristics (Table 1).

Correlation between romantic relationship satisfaction and variables

The degree of romantic relationship satisfaction of the subjects was positively correlated with self-esteem ($r=0.53$, $p<0.001$) and negatively correlated with avoidance attachment ($r=-0.40$, $p<0.001$), anxiety attachment ($r=-0.42$, $p=0.008$), and sex attitude ($r=-0.19$, $p=0.008$). In other words, a higher degree of self-esteem, lower avoidance and anxiety attachment, and more conservative sexual attitudes all led to higher satisfaction in romantic relationships (Table 3)

Table 3. Correlations Coefficient among the Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Avoidance attachment</th>
<th>Anxiety attachment</th>
<th>Sexual attitude</th>
<th>Self-esteem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ ($p$)</td>
<td>$r$ ($p$)</td>
<td>$r$ ($p$)</td>
<td>$r$ ($p$)</td>
</tr>
<tr>
<td>Romantic relationship satisfaction</td>
<td>-.40 ($&lt;.001$)</td>
<td>-.42 ($&lt;.001$)</td>
<td>-.19 (.008)</td>
<td>.53 ($&lt;.001$)</td>
</tr>
</tbody>
</table>

Influencing factors on romantic relationship satisfaction

As a result of verifying the multicollinearity before performing regression analysis, the variance expansion index (VIF) among the variables is 1.140 to 1.561, which is less than 10, which can be considered free of autocorrelation. In addition, the Durbin-Watson statistic that confirms the independence among the error terms was found to be 1.594, which satisfies the assumption of independence. A total of four independent variables were used to identify the determinants of romantic relationship satisfaction: adult attachment (avoidance attachment and anxiety attachment), sexual attitude, and self-esteem. The stepwise regression analysis
The results are as follows: self-esteem ($\beta=0.330$, $p<0.001$), anxiety attachment ($\beta=-0.237$, $p<0.001$), and avoidance attachment ($\beta=-0.209$, $p=0.002$). The exploratory power of the three variables 34.7%, and the most influential variable was self-esteem (Table 4).

Table 4. Influencing Factors on Romantic Relationship Satisfaction

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>$\beta$</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-2.334</td>
<td>-5.371</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Self-esteem</td>
<td>.275</td>
<td>.330</td>
<td>4.574</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Anxiety Attachment</td>
<td>-.213</td>
<td>-.237</td>
<td>-3.601</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Avoidance Attachment</td>
<td>-.246</td>
<td>-.209</td>
<td>-3.166</td>
<td>.002</td>
</tr>
</tbody>
</table>

F=33.875, $p<.001$, Adj R$^2=.347$

Discussion

In this study, the romantic relationship satisfaction level of the university students was 2.59 points on a scale within a range from 1 to 5. This score was lower than the average of 3.47 points of the local university students, which was measured with the identical measurement tool[17].

As a result of examining the correlation between university students’ romantic relationship satisfaction and their variables, it was found that adult attachment had a negative correlation with romantic relationship satisfaction. This result is consistent with Choi and Kim[17] and Yildiz, Cokamay, and Artar[18]. In general, the two dimensions of adult attachment, avoidance attachment and anxiety attachment, are viewed as unstable attachment, and there tend to be more relationship problems as the degree of adult attachment grows stronger[19]. Anxiety attachment in this study had a stronger negative correlation with romantic relationship satisfaction than did avoidance attachment, which is similar to the results in the previous literature[17].

People with high anxiety attachment tend to focus on their stress, reflect on negative thoughts, and emotion-centered coping strategies. On the other hand, those with a high level of avoidance attachment use strategies that cognitively and behaviorally distance themselves from the cause of stress[20].

The university students’ sexual attitude and romantic relationship satisfaction were found to have a negative correlation, which was consistent with the prior result of Hendrick, Hendrick, and Reich[21] that more conservative sexual attitude leads to higher romantic relationship satisfaction. Conversely, a study of unmarried men and women with sexual experience reports a significant positive correlation between sexual consciousness and romantic relationship satisfaction[13], showing no consistency in the correlation between sexual attitude and romantic relationship satisfaction. In the future, if one establishes a strategy to maintain a satisfactory relationship by identifying the attitude of individuals, he or she will be able to increase satisfaction on the relationship.

Furthermore, romantic relationship satisfaction is higher in the group with high self-esteem[22], which is consistent with the results of this study. Through these results, one will be able to enhance romantic relationship satisfaction by turning negative sexual attitude into positive sexual attitude when establishing a strategy to maintain a satisfactory romantic relationship.

After analyzing the effects on romantic relationship satisfaction in the study, self-esteem, ($\beta=0.330$, $p<0.001$), anxiety attachment ($\beta=-0.237$, $p<0.001$), and ($\beta=-0.209$, $p=0.002$) explain 34.7% of romantic relationship satisfaction. Among them, self-esteem was the most influential variable on relationship satisfaction. It can be interpreted that those who love themselves and feel valued maintain satisfactory relationships with their partners[23]. There are several factors in the characteristics which people with low self-esteem exhibit in relationships. First, they need more acceptance and incorrectly perceive others’ attitude. These people believe that they need to live up to the specific standards they have set for themselves or need to have valuable traits to be loved and accepted by important people, so they underestimate the positive attitudes their partners have toward themselves or evaluate less of their partners’ attitude[11]. If the partner’s continued acceptance is suspected, he or she could distance himself or herself from the partner, devalue romantic relationships,
be unnecessarily disappointed in them, and end the relationship in dissatisfaction [24].

Anxiety and avoidance attachment, which are forms of unstable attachment among adult attachments, were the second and third factors that explained the relationship satisfaction of the university students. Hazan and Shaver[7] argue that adult attachment is the one of the most well-known variables in predicting relationship satisfaction and is related with romantic relationships in adulthood. Their findings demonstrate that positive emotions as happiness and trust are usually reported in relationships in the case of stable attachment, whereas instable attachment types such as avoidance or anxiety feel a lot of negative emotions such as discomfort or jealousy in relationships.

The overall results suggest that it is necessary for university students, in order to improve the romantic relationship satisfaction, to have a positive and active life attitude toward their own lives so that they can stablish positive sexual attitudes and enhance their self-esteem.

Conclusions

In this study, self-esteem, anxiety attachment, and avoidance attachment were significant predictors that influence university students’ romantic relationship satisfaction and the three variables’ explanatory power was 34.7%. Based on these results, it is necessary for university students to develop a sense of self-respect and experience the process of exchanging positive feelings with their partners in order to improve their satisfaction with romantic relationships.

This study is meaningful in that it takes account sexual attachment variables, a new concept in nursing, into account to provide nursing intervention to enhance healthy relationship satisfaction of university students.

Ethical Clearance: Not required

Source of Funding: Self

Conflict of Interest: Nil

References


Video Self Modelling - An Intervention to Study Bell’s Palsy through Kinect Azure: A Research Protocol

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Abstract

Background: Bell’s palsy is the common acute mono-neuropathy, and is commonly associated with facial paralysis or weakness of facial nerve. It is unilateral facial nerve paresis or paralysis. The cause of Bell’s palsy is suspected to be herpes simplex virus infection of nerve. The nerve get swollen because of this viral infection and is compressed in its canal as it passes through the temporal bone. Grading systems for the assessment of movements and asymmetry of face in Facial palsy are divided into computer-based and traditional grading systems. The program that uses Kinect Azure provides assessment method for evaluation of asymmetry of face at rest and the rating of facial palsy during voluntary activity of various areas over face. This study aims to investigate the intervention of facial palsy by video self modelling with the use of Kinect Azure.

Methods: 20 participants will be selected. Each group will include 10 subjects. Group A will receive conventional treatment, electrical muscle stimulator(EMS), and visual feedback.

Group B (Experimental group) will receive conventional treatment, electrical muscle stimulator and video self modelling. Each participant would be presented with their own videotape of video self-modelling, which included the best attempts at their evenest acts (smiles). Following 2 weeks of tape viewing the actions will be assessed. The outcome of the treatment will be assessed by Kinect Azure.

Discussion: Traditional methods for documentation of treatment effect have been through scales and questionnaires which at times are little complex and also difficult for patients to interpret. Hence this experimental and comparative study aims at focusing on the effective use of Kinect to document outcome for bell’s palsy.

Key words: Bell’s palsy, physical therapy, Kinect.

Introduction

Bell’s palsy is the common acute mono-neuropathy, and is commonly associated with facial paralysis or weakness of facial nerve. It is unilateral facial nerve paresis or paralysis. This disorder causes complete or partial inability of the paralysed side of face to voluntarily move facial muscles. The paresis or paralysis of face in Bell’s palsy result in inability to close the eyelid and temporary oral incompetence resulting in possible injury to the eye. (4) Annual incidence of palsy is 15 to 30 per 100,000 people, with equal numbers of women and male affected. Any side of the face has no predilection. The palsy of Bell was identified in patients of all ages, with peak incidence in the 40s. It occurs more frequently in diabetes patients and pregnant females. (2)

The cause of Bell’s palsy is suspected to be herpes simplex virus infection of nerve. The nerve get swollen because of this viral infection and is compressed in its canal as it passes through the temporal bone. (3) Symptoms typically begin in the first week, and then gradually resolve over 3 to 3 months. It is in patients with diabetes, and while it can affect people of any age, incidence peaks in 40s. (5) Paresis is the most disturbing symptom of Bell’s palsy; up to three quarters of affected
people assume they have had a stroke or an intracranial tumour. The palsy frequently starts unexpectedly and progresses quickly, with maximum facial weakness occurring within two days. Hyperacusis, reduced tear production, and altered taste may be associated with symptoms. Patients may also report otalgia or aural fullness, and facial or retroauricular pain, usually mild and preceding palsy. Severe pain indicates herpes zoster virus, and can lead to a vesicular eruption and progression to Ramsay Hunt syndrome. Features can lead to a mild polynoepathy. A gradual progressive paralysis with other cranial nerve defects or headache increase the neoplasm possibility (4).

Video self-modelling is used with movements of face affected by facial nerve palsy (Lower Motor Neuron type). Although self-modeling patients view video clips of themselves engaging only in the correct, adaptive behavior and so the treatment relies on the patient being able to generate the desired form of behavior at least once. The action, or movement pattern, is recorded, edited into a short set of images by only choosing the best presentation and then given to the patient to watch again before replaying. Analyzing one’s best attempts at a desired reaction has been used to promote motor learning in a variety of therapeutic settings, and it improves the degree of success that integrates previously unachievable abilities. vBecause an successful smile provides the audience with a powerful emotional input, this study was designed to examine the use of video self-modelling as a method of adapting the smiles after facial nerve palsy. (5)

Evaluation of paralysis of face and quantitative grading of asymmetry is important to measure the severity of the disorder as well as to monitor its progression or improvement. As such, a precise quantitative grading system is needed which is easily understand, cheap and has minimum variability. As there is clearly a need for a clinically feasible tool that can assess the severity of the disease and the resultant loss of function from Facial palsy and can also calculate the efficacy of medical care or surgery. Such a tool should be qualitative, standardized, depending little or no on the observer, and cost-efficient.

Grading systems can be divided into conventional and computer based grading systems for determining facial gestures and facial asymmetry in facial palsy (6). Modern approaches include the House-Brackmann grading system (HBGS)-The House and Brackmann grading system is recommended as a common standard for determining the degree of facial paralysis and is a clear and accurate method for evaluating facial function. (7) Functional disability index – It is a disease-specific, self-reporting functional status instrument that provides an essential component for the evaluation of citizens with facial neuromuscular disorders. (8) Many programs include Burres-Fisch, Nottingham, Sunnybrook, and many others. Computer-based FP grading systems including video recording and image processing are also proposed. (6)

Kinect Azure provides assessment method for evaluation of asymmetry of face at rest and the rating of facial palsy during voluntary activity of various areas over face. This study aims to investigate the intervention of facial palsy by video self modelling with the use of Kinect Azure.

**Aim**

This study aims to analyze the intervention of Bell’s palsy by visual self-modelling using Kinect Azure.

**Methodology**

**Study setting:**

The trial will be carried out in HumEn research lab and Neuro Physiotherapy Department of Ravi Nair Physiotherapy College, DMIMS, Sawangi(Meghe), Wardha, Maharashtra, India, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University.

**Study Design and Sample Size:**

The design of the study is a single blinded randomized controlled trial of a Kinect Azure for individual diagnosed with bell’s palsy and it is an experimental and comparative study.

By using purposive sampling we will select 20 subjects(n=20) and will include 10 subjects in each groups (Group A and B). All topics will be clarified in detail about the research and a written informed consent will be taken. Group A will receive conventional
treatment, electrical muscle stimulator (EMS), and visual feedback. Group B (Experimental group) will receive conventional treatment, electrical muscle stimulator, and video self modelling. All topics will be clarified in detail about the research, and a written informed consent will be taken. The schedule of enrollment, interventions, and assessments of the study is illustrated in Figure 1.

<table>
<thead>
<tr>
<th>STUDY PERIOD</th>
<th>Enrolment</th>
<th>Allocation</th>
<th>Post-allocation</th>
<th>Follow-up test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TIMEPOINT</strong></td>
<td></td>
<td></td>
<td>Intervention</td>
<td>Post-test</td>
</tr>
<tr>
<td><strong>ENROLLMENT:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eligibility screen</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td>X</td>
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<td></td>
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<tr>
<td>Allocation</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INTERVENTIONS:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>{Conventional}</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>{video self modelling}</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ASSESSMENTS:</strong> House-Brackmann Scale, Facial disability index</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcomes measure: Kinect Azure</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1 Schedule of enrolment, interventions and assessments.**

Participants

The Inclusion Criteria for the participants are as under:

1. Those who are willing to participate.
2. Patient affected by acute onset paralysis without detectable cause
3. Those with Idiopathic facial paralysis
4. Rehabilitation treatment carried out at our own hospital
5. Those who have unilateral facial paralysis-LMN type
6. Those who are between 20 to 42 years of age.
7. Those who scored above grade 3 on House brachman scale
8. Those have Normal superficial and deep sensation

The Exclusion Criteria for the participants are as under:
1. Those who are not willing to participate
2. Those with any type of facial fracture
3. Those with Known traumatic, inflammatory, Neoplastic pathology of facial nerve
4. Those with Bilateral facial paralysis
5. Those with UMN type facial palsy
6. Those who have Disease of central or peripheral nervous system
7. Those who scored below grade 3 on House brachman scale.
8. Those with Recent head injury
9. Those who have Psychiatric disease

PARTICIPANT TIMELINE:

The study duration is of 6 months and intervention duration is 2 weeks.

Assessment will be done on 1st day of visit following 2 weeks of tape viewing the actions will be reassessed.

RECRUITMENT: The neurologists and health care practitioners working under DMIMSU are invited to refer the prospective patients to our inpatient department (IPD). Regular visit to Neuromedicine, Neurosurgery wards will be done and contact will be maintain with doctors, record maintaining office for cases that will enrolled in hospital so that can be taken for study. The patients who are already undergoing treatment in our IPD and diagnosed with facial palsy will be assessed for the eligibility in the study as per the inclusion and exclusion criteria. Informed patient consent will be taken before allocation and after elaborating the purpose, nature, procedure, benefits and effects of the intervention.

Implementation:

Selection of the participants will be supervised by the research coordinator and principal investigators.

Blinding:

Tester(s) will be blinded to assign the subjects to the group. To ensure blinding, subjects will be mandated not to reveal any details of their treatment to the tester.

Study procedure:

The participants will be categorized into 2 groups:

**Group A:** (Conventional physiotherapy)
The participants in this group will undergo 1 hour of conventional physiotherapy program daily, 5 days per week for 2 weeks. It will be performed by a physiotherapist in IPD. It will comprise of electrical muscle stimulator, visual feedback, facial exercises.

**Group B:** (Video self modelling combined with conventional physiotherapy) The participants in this group will undergo 30 minutes of conventional physiotherapy and 30 min of video self modelling based physiotherapy daily for 5 days per week for 2 weeks provided by physiotherapist in IPD. Each participant would be presented with their own videotape of video self-modeling, which included the best attempts at their evenest acts (smiles). In the following way we will make a videotape. A mobile video camera will be at a constant distance from the subject matter and set to run at session start. Subjects would be asked to complete a series of 5 smiles that included both their normal “everyday” (nonlinear) smile and their best (linear) “adapted” smile. The entire tape will be reviewed after a session, and 2 or 3 of the best smiles will be recorded using video editing software. Following 2 weeks of tape viewing the actions will be assessed.
The outcome of the treatment will be assessed by Kinect Azure from first day and after 2 weeks of intervention.

Outcomes

Primary outcome measures:

1) House-Brackmann Scale- For assessment of degree of facial paralysis.

2) Facial Disability Index used as an initial assessment tool and as an monitoring instrument to view the outcome of intervention.

Secondary outcome measure:

Kinect Azure

DATA COLLECTION AND MANAGEMENT

Data collection

The assessment data will be collected from a pre-established spreadsheet with the baseline characteristics variable. Testing data will be put into a secure REDCap database. The nonelectronic data, such as hard copies of assessment forms, signed consent forms, etc. will be stored securely in the study setting. The employment of regular feedback concerning adherence and reminder phone calls (for attending the treatment) will be done.

Data management:

Data collection and documentation will be done under the guidance of the principal investigators. The study documentation will be evaluated thoroughly for accuracy. The Excel spreadsheet will be released at the end of the study to an allocation blinded statistician for conducting the necessary analysis, following which unblinding of the groups will be done. Checklists are used to prevent missing data due to the improper staff procedure.

Statistical Analysis Plan

Therapy induced changes in the primary outcome measures will be analysed via mixed-effects linear models across ‘time’ (pre-intervention vs post-intervention) and ‘group’ (Experimental vs control). The comparison will be done between the two groups using t-tests for the demographic measures and initial scores on outcome measures. For the interpretation of the results, we will significant differences. Significance will be set at P less than 0.05. The results will be accounted for as per the CONSORT guidelines.

BIAS

Our study will have a low degree of selection bias (Oculus Quest). Measures will be taken to prevent attrition bias by giving reminder calls before each intervention and by giving transportation aids to those who require it. Thus we anticipate a low percentage of dropouts.

Discussion

Our study aims to estimate efficacy of video self-modelling compared to conventional physiotherapy in individuals with Bell’s palsy. Physical therapy has played a significant part in Bell’s palsy management. Rehabilitation appeared effective in recovering facial symmetry, reducing paresis severity by 0.6 grades on the HB scale, and controlling synkinesis. Physiotherapy rehabilitation of an adapted (more symmetrical) smile was investigated by Dr Susan E in FNP subjects 1 year after the start, using video self-modeling (video replay of only the best adapted smiles) and implementing intentions (preplanning adapted smiles for specific situations and concluding that reaction time (RT) for the initiation of adapted smiles was 224 ms faster, adapted smiles were completed 544 ms faster, adapted smiles had higher overall quality, movement control and symmetry ratings, and Facial Disability Index scores also improved. His study supports these techniques of rehabilitation to maximize the quality of the smiles following facial nerve palsy. Kinect platform can be used to develop low cost approaches to measure the movement aspects objectively.

Research and development prospects and future therapeutic applications work with the Kinect are comprehensive. This will help to make diagnostic and prognostic evaluations.

Ethical Approval And Dissemination

Ethical approval will be taken from institutional ethical committee. The DMIMS which will fund research and the subjects which will participate in the study will be able to access the research’s main findings. For the
enrolled subjects, data held safely for a minimum of five years. Once data collection is complete, a completion report will be produced for statistical analysis and sent for publication after review by institutional research cell.

**Patient Consent**

Principal Investigators will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality.

**Confidentiality**

The study program will be explained to the participant and one of his/her relative, and the principal investigator will take personal information. The consent form will include the confidentiality statement and signatures of the principal investigator, patient and 2 witnesses. If required to disclose some information for the study, consent will be taken from the patient with complete assurance of his confidentiality.

**Author’s contribution**

DJ suggested the design of the study. MD and DJ led to the creation and design of the study. MD wrote the manuscript of this article. MD and DJ read and approved the final manuscript for publication.

**Declaration of interests**

The authors declare no conflicting interest.

**Funding**

No direct support will be taken for funding this research from any public and private organizations. The Department of Physiotherapy under Datta Meghe Institute Of Medical Sciences, Deemed to be University, will provide the necessary material for the research.

**References**

The Association between Teeth Loss and Oral Health Problems

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1Assistant Professor in Oral surgery, University of Science and Technology of Fujairah, UAE., 2Assistant Professor Department of Oral surgery, Sebha University, Libya, 3Assistant Professor, Departments of Orthodontic, Sebha University, Libya, 4Associate Professor in Periodontics, University of Science and Technology of Fujairah. UAE.

Abstract

Background: The teeth play various functional roles, from the most basic functions to more subtle functions. One of the most tragic events that can happen to the teeth are loss it, as a result trauma or dental diseases. Tooth loss has esthetic, functional, positional and psychological impacts on the life of individuals.

Objectives: To investigate the association between tooth loss and oral health problems among partially and completely edentulous patients. Method: (Questionnaire and Clinical Examination). Self-administered questionnaire was distributed to the patients to collect information relating to demography and cause of tooth loss, in addition to mastication and speech state after tooth loss. Four clinical conditions including supra-eruption, drifting teeth, temporomandibular disorders and bone resorption were used to recognize the effects of missing teeth. Patients seeking dental treatment at General Hospital, Morzuk, Libya were recruited for the study. Criteria included age of 16 years and above with one or more missing teeth except for third molars. Results: altogether 58 participants, 31 (53.4 %) were males while 27 (46.6%) were females. The participants were aged 16–>45 years, among them, 44 patients (75.9%) are partially edentulous, and 14 patients (24.1%) were completely edentulous. The patients with complete teeth loss and the patients with missing both the anterior and posterior teeth are most groups suffer problems among patients who involved in the study. patients who loss posterior teeth are the lowest group had, suffer of health problems due to loss of teeth among all of them.

The general relationship between tooth loss and the oral health problems was positive and strong. Conclusions: there are statistically significant relationship between tooth loss and oral health problems, where the strong value of this relationship (0.614) and significance within less than (0.05).

Key words: Tooth loss, consequences of tooth loss, causes of tooth loss.

Introduction

The teeth play various functional and aesthetic roles as essential daily activities from the most basic functions like eating and speaking to subtler functions related to good appearance1. Teeth are anchored in the jaws by the periodontal ligament. This ligament connects the cervix (neck) of the tooth, at the junction between the crown and root, to the gingiva. Below that, the ligament connects the outer layer of the tooth root (cementum), to the adjacent bone (jawbone). The jawbone of the maxilla and mandible that support the teeth is the alveolar bone2.

One of the more dramatic discoveries in the medical sciences in the twentieth century has been the realization that tooth loss is not an inevitable consequence of aging, but the result of trauma or dental diseases3. Tooth loss continues to be a major problem in clinical dentistry and has received significant attention in everyday dental practice4.

In most developing countries, the main reason for people to seek for dental care is pain that has become intolerable after a long period of “wait-and-see”5-6. Because of the delay in seeking treatment, the patients present with destroyed teeth that is impossible to treat
by the conventional restorative procedures. Likewise, insufficient finances limit restorative and rehabilitative dental treatment as well as shortage of professionals and dental materials. Therefore, we can assume that, many patients have a large number of missing teeth. The absence of one or more natural teeth often results in disability. This can trigger several positional and functional changes such as impairment in speech, mastication ability and integrity of dental arch.

Studies have shown that, tooth loss can have a substantial effect on the emotions as well as oral health and function. In the past, many patients felt that tooth loss was inevitable and were, to a certain extent, prepared for that eventuality.

Today, patients’ expectations have changed and many see tooth loss as a negative event. Patients may suffer real or perceived detrimental effects following the loss of one or more teeth.

**Problem Of Research**

Recent studies have confirmed the findings of many researchers that, both functional and psychological compromises as well as positional changes are following tooth loss if unrestored.

These positional changes may be of sufficient magnitude to complicate restorative treatment. Therefore, the desire of patients to have an excellent prosthesis for their missing teeth may pose dental technician with many challenges to achieve ideal function and best esthetics.

Many studies are available exhibiting the causes of tooth loss, but comparatively there are fewer studies documenting the effects or consequences of tooth loss, literature is scarce about the consequences of tooth loss in our country.

There is however, paucity of information on whether patients or people with missing teeth are aware of the side effects of tooth loss on them or on the remaining teeth.

**This study will help :**

- To understand the causes and consequences of tooth loss among our patients.
- Demonstration about the relationship between tooth loss and oral health problems.
- Encourage our patients for replacing their lost teeth.

**Objectives**

- To recognize the causes and effects of tooth loss.
- To investigate the association between loss teeth and oral health problem.
- To determine functional and positional changes that following tooth loss.
- To prioritize restoration and replacement of missing teeth to meet functional demands, and ensure quality dental services for all citizens.

**Materials and Method**

(Questionnaire and Clinical Examination)

Self-administered questionnaire was distributed to the patients to collect information relating to demography and cause of tooth loss, in addition to mastication and speech state after tooth loss.

Four clinical conditions including supra-eruption, drifting teeth, temporomandibular disorders and bone resorption were used to recognize the effects of missing teeth.

Patients seeking dental treatment at General Hospital, Morzuk, Libya were recruited for the study.

Criteria included age of 16 years and above with one or more missing teeth except for third molar.

**A. Study Design.**

A short-term study design

**B. Study Area.**

This study conducted in the prosthetic clinic at the General Hospital of Morzuk, Libya. The location of this hospital is in the city center beside the modern mosque along the main road.

**B. STUDY SAMPLE.**

The study was conducted among the partially and
completely edentulous patients who attended dental clinic at the hospital for refers or received various oral health services.

C. INCLUSION CRITERIA.

- The people who have missing teeth, either partially or completely edentulous.
- Age is 16 years old and above.

D. EXCLUSION CRITERIA

- Any subject who have loss of third molars alone (considered as complete dentition).
- The patients who have teeth extraction for orthodontic purposes (treatment objectives).

E. ETHICAL CONSIDERATIONS

Ethical clearance for this study was obtained from Dental Prosthetic Department of the Medical Technology College in Sabha University.

The study also was approved by the administration protocol of General Hospital of Morzuk, Libya. In addition, every 23 participant was informed of his or her right to refuse participation or to withdraw from the study at any moment.

TOOLS AND TECHNIQUE OF COLLECTION OF DATA.

1. QUESTIONNAIRE (OPEN-CLOSE):

- Self-administered open-close questionnaire used to collect information from the patients. The questionnaire contained 13 questions, one was dichotomous, and one question had space for the participant to write the answer.

The questions in the beginning enquired about the personal information such as age, gender, economic and education status. Thereafter, the subjects questioned about causes and duration of tooth loss, in addition to some effects that happened after tooth loss.

The subjects were of four age groups ascending from 16 to more than 45 years of age (16-25 years, 26-35 years, 36-45 years, and >45 years).

2. CLINICAL EXAMINATION:

- Clinical examination conducted by using examination tools: dental chair, mouth mirror, tweezers and periodontal probe.

The examination included clinical variables such as type of edentulousness (partial or complete), position and type of missing teeth. In addition to the consequences of tooth loss on patients’ wellbeing, such as temporomandibular disorder, drifting of remaining teeth, overeruption of unopposed teeth and bone loss (resorption).

To determine the position and type of missing teeth, we used graphical representation to represent the upper and lower teeth. In addition, we have also used it to determine whether the teeth have drifting (inclining) or overeruption.

Results:

Altogether 58 participants, 31 (53.4 %) were males while 27 (46.6%) were females. The participants were aged 16 – >45 years, among them, 44 patients (75.9%) are partially edentulous, and 14 patients (24.1%) were completely edentulous. Among the study group, the patients with complete tooth loss, patients with missing both anterior and posterior teeth were suffering with more problems. The least suffering was those who lost posterior teeth.

Many oral health problems had shown high average occurrence on T-Test and strong relationship on Pearson correlation. The general relationship between tooth loss and the oral health problems was positive and strong.

Conclusions

There is a statistically significant relationship between tooth loss and oral health problems, where the strong value of this relationship was (0.614), and the level of significance was less than (0.05).

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A short-term study design

B. STUDY AREA.

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To determine the position and type of missing teeth, we used graphical representation to represent the upper and lower teeth. In addition, we have also used it to determine whether the teeth have drifting (inclining) or overeruption.

Data Analysis

All Data was analyzed by using Statistical package for Social Science SPSS (Version 18)

- Analysis included one sample T-Test and Pearson Correlation to find the correlation between the tooth loss and oral health problems. In the analysis, the tooth loss category was used as independent variable; it is divided into four groups:

  · Missing anterior teeth only.
  · Missing posterior teeth only.
  · Missing both anterior and posterior teeth.
  · Complete teeth loss.

The dependent variable was that problems who followed the tooth loss: positional changes (overeruption and drifting teeth), functional changes (low mastication, poor aesthetic and speech distortion), bone loss (resorption) and emotional effects of tooth loss.34 significance (0.000). Similarly, increase in tooth loss led
to increase in low mastication or low chewing ability.

As for the relationship between tooth loss and speech distortion was positive with medium-strength, the level of significance reached (0.002).

With increase of teeth loss, the distortion of speech also increased.

The relationship between tooth loss and aesthetic problems (poor appearance) was positive with slight-strength correlation (1.261), the level of significance less than (0.05).

With increase of teeth loss, the aesthetic problems also increases. The problem of drifting teeth showed a negative low correlation (reverse relationship) the level of significance was (0.037).

Increase in tooth loss led to a decrease in drifted teeth.

As for the problems of the negative emotional effects and bone loss (resorption) are showed positive correlation but low strength (weak), but indicative of the level of significance higher than (0.05), that correlation is not significance in both problems. While, the overeruption of unopposed teeth is showed a negative correlation (reverse relationship), but the significant is higher than (0.05). Therefore, the correlation is not significant.

The general relationship between tooth loss and the oral health problems (all previous problems), the correlation value was (0.614), and the level of significance was (0.000), which is less than (0.05). It is a positive and strong correlation, and with increasing teeth loss, there is also an increase in oral health problems.

**Result**

Fifty-eight patients consisting of 31 male (53.4 %) and 27 (46.6%) female participated in the study. The ages ranged from 16 to more than 45 years. The majority of participants were within the age group 26-35 years (29.3%). About half of the respondents (48.3%) had academic education or more.

About 18 (31%) of participants were their answers about first experience with tooth loss are (5-10 years ago).

Among the participants, 44 patients (75.9%) are partially edentulous, and 14 patients (24.1%) were completely edentulous. Of those with partially missing teeth some of them had, missing teeth in anterior only 16 (27.6%), and some had missing teeth only in the posterior region 14 (24.1%), while others had missing teeth in both anterior and posterior regions 14 (24.1%).

**Causes of tooth loss**

The most common cause of tooth loss in this study was dental caries 27 (46.6%), followed by Periodontal disease 20 (34.5%), trauma was responsible for 9 (15.5%) cases of tooth loss, and lastly, just two patients (3.4%) had congenitally missing teeth.

<table>
<thead>
<tr>
<th>The causes</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dental caries</td>
<td>27</td>
<td>46.6</td>
<td>46.6</td>
<td>46.6</td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>20</td>
<td>34.5</td>
<td>34.5</td>
<td>81.0</td>
</tr>
<tr>
<td>Congenitally</td>
<td>2</td>
<td>3.4</td>
<td>3.4</td>
<td>84.5</td>
</tr>
<tr>
<td>Trauma</td>
<td>9</td>
<td>15.5</td>
<td>15.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

The results of T-test about the consequences of tooth loss.
PATIENTS WITH MISSING ANTERIOR TEETH ONLY

Table 2: The health problems which suffers of it patients with missing anterior teeth

<table>
<thead>
<tr>
<th>N</th>
<th>Oral health problems</th>
<th>No</th>
<th>Yes</th>
<th>Mean</th>
<th>S.D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Temporomandibular disorder (TMD)</td>
<td>16 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>2</td>
<td>Overeruption</td>
<td>16 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>3</td>
<td>Drifting teeth</td>
<td>12 (75%)</td>
<td>4 (25%)</td>
<td>1.25</td>
<td>0.447</td>
<td>0.041</td>
</tr>
<tr>
<td>4</td>
<td>Low chewing ability</td>
<td>15 (93.8%)</td>
<td>1 (6.2%)</td>
<td>1.063</td>
<td>0.25</td>
<td>0.000</td>
</tr>
<tr>
<td>5</td>
<td>Poor aesthetical effects</td>
<td>1 (6.2%)</td>
<td>15 (93.8%)</td>
<td>1.938</td>
<td>0.25</td>
<td>0.000</td>
</tr>
<tr>
<td>6</td>
<td>Speech distortion</td>
<td>3 (18.8%)</td>
<td>13 (81.2%)</td>
<td>1.813</td>
<td>0.403</td>
<td>0.007</td>
</tr>
<tr>
<td>7</td>
<td>Bone loss (resorption)</td>
<td>1 (6.2%)</td>
<td>15 (93.8%)</td>
<td>1.938</td>
<td>0.25</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>Negative emotional effects</td>
<td>6 (37.4%)</td>
<td>10 (62.5%)</td>
<td>1.313</td>
<td>0.946</td>
<td>0.44</td>
</tr>
</tbody>
</table>

From previous table, it is clear that, these patients were exposure significantly to aesthetic problems (poor appearance) and bone loss (resorption) due to loss of anterior teeth, where the mean was in both of them (1.938) and standard deviation was (0.25), the level of significance is less than (0.05).

As well as they had clearly speech distortion, where the average (1.813) and standard deviation (0.403) the connotation level of significance less than (0.05).

As for the problem of temporomandibular disorder (TMD) and overeruption of unopposed tooth, did not founded ever among them, where the mean was in both of them (1) and standard deviation was (0.000), the level of significance is less than (0.05).

PATIENTS WITH MISSING POSTERIOR TEETH ONLY

Table 3: The health problems that suffers of it patients with missing posterior teeth

<table>
<thead>
<tr>
<th>N</th>
<th>Oral health problems</th>
<th>No</th>
<th>Yes</th>
<th>Mean</th>
<th>S.D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Temporomandibular disorder (TMD)</td>
<td>13 (92.9%)</td>
<td>1 (7.1%)</td>
<td>1.071</td>
<td>0.267</td>
<td>0.000</td>
</tr>
</tbody>
</table>


Cont... Table 3: The health problems that suffers of it patients with missing posterior teeth

<table>
<thead>
<tr>
<th></th>
<th>Oral health problems</th>
<th>No</th>
<th>Yes</th>
<th>Mean</th>
<th>S.D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Overeruption</td>
<td>6 (42.9%)</td>
<td>8 (57.1%)</td>
<td>1.571</td>
<td>0.514</td>
<td>0.612</td>
</tr>
<tr>
<td>3</td>
<td>Drifting teeth</td>
<td>4 (28.6%)</td>
<td>10 (71.4%)</td>
<td>1.714</td>
<td>0.469</td>
<td>0.111</td>
</tr>
<tr>
<td>4</td>
<td>Low chewing ability</td>
<td>11 (78.6%)</td>
<td>3 (21.4%)</td>
<td>1.214</td>
<td>0.426</td>
<td>0.026</td>
</tr>
<tr>
<td>5</td>
<td>Poor aesthetical effects</td>
<td>11 (78.6%)</td>
<td>3 (21.4%)</td>
<td>1.214</td>
<td>0.426</td>
<td>0.026</td>
</tr>
<tr>
<td>6</td>
<td>Speech distortion</td>
<td>14 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>7</td>
<td>Bone loss (resorption)</td>
<td>4 (28.6%)</td>
<td>10 (71.4%)</td>
<td>1.7143</td>
<td>0.469</td>
<td>0.111</td>
</tr>
<tr>
<td>8</td>
<td>Negative emotional effects</td>
<td>10 (71.4%)</td>
<td>4 (28.6%)</td>
<td>1.143</td>
<td>0.663</td>
<td>0.065</td>
</tr>
</tbody>
</table>

The previous table shown that, A few of these patients complain of low chewing ability and aesthetic problems (poor appearance) due to the loss of posterior teeth, where the average of answers was (1.214), the standard deviation (0.426) and the moral level of significance less than (0.05).

The temporomandibular disorders are very slightly among them, where the average was (1.071) and standard deviation was (0.267), and within less significance of (0.05).

As for the problems overeruption and the drifting teeth and the negative emotional effects and the bone loss (resorption) the indicative of significance level of these problems was higher than (0.05), so can consider that the problem does not exist among them.

PATIENTS WITH MISSING BOTH ANTERIOR AND POSTERIOR TEETH

Table 4: The health problems which suffers of it patients with missing both anterior and posterior teeth

<table>
<thead>
<tr>
<th>N</th>
<th>Oral health problems</th>
<th>No</th>
<th>Yes</th>
<th>Mean</th>
<th>S.D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Temporomandibular disorder (TMD)</td>
<td>13 (92.9%)</td>
<td>1 (7.1%)</td>
<td>1.071</td>
<td>0.267</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>Overeruption</td>
<td>3 (21.4%)</td>
<td>11 (78.6%)</td>
<td>1.786</td>
<td>0.426</td>
<td>0.026</td>
</tr>
<tr>
<td>3</td>
<td>Drifting teeth</td>
<td>1 (7.1%)</td>
<td>13 (92.9%)</td>
<td>1.929</td>
<td>0.267</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>Low chewing ability</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
<td>1.643</td>
<td>0.497</td>
<td>0.302</td>
</tr>
<tr>
<td>5</td>
<td>Poor aesthetical effects</td>
<td>2 (14.3%)</td>
<td>12 (85.7%)</td>
<td>1.857</td>
<td>0.363</td>
<td>0.003</td>
</tr>
<tr>
<td>6</td>
<td>Speech distortion</td>
<td>10 (71.4%)</td>
<td>4 (28.6%)</td>
<td>1.286</td>
<td>0.469</td>
<td>0.111</td>
</tr>
<tr>
<td>7</td>
<td>Bone loss (resorption)</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
<td>2</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>8</td>
<td>Negative emotional effects</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
<td>1.427</td>
<td>0.852</td>
<td>0.759</td>
</tr>
</tbody>
</table>
The previous table shown that, the exposure to bone loss (resorption) is very high among these patients, and they suffering from it clearly, where the average (2) standard deviation (0.000) and the level of significance less than (0.05).

Furthermore, the degree of exposure of these patients to drifting teeth, aesthetic problems (poor appearance) and overeruption of unopposed teeth was high, within significance less than (0.05).

While the degree of exposure of these patients to temporomandibular disorder (TMD) is very low, and the level of significance is less than (0.05). Moreover, about other problems in the table, it have the highest significance of (0.05).

PATIENTS WITH COMPLETE EDENTALOUS (COMPLETE TEETH LOSS)

Table 5: The health problems which suffers of it patients with complete teeth loss.

<table>
<thead>
<tr>
<th>N</th>
<th>Oral health problems</th>
<th>No</th>
<th>Yes</th>
<th>Mean</th>
<th>S.D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Temporomandibular disorder (TMD)</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
<td>2</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>2</td>
<td>Overeruption</td>
<td>14 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>3</td>
<td>Drifting teeth</td>
<td>14 (100%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>4</td>
<td>Low chewing ability</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
<td>2</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>5</td>
<td>Esthetical effect</td>
<td>2 (14.3%)</td>
<td>12 (85.7%)</td>
<td>1.857</td>
<td>0.363</td>
<td>0.829</td>
</tr>
<tr>
<td>6</td>
<td>Speech distortion</td>
<td>0 (0%)</td>
<td>14 (100%)</td>
<td>2</td>
<td>0.000</td>
<td>----</td>
</tr>
<tr>
<td>7</td>
<td>Bone loss (resorption)</td>
<td>1 (7.1%)</td>
<td>13 (92.9%)</td>
<td>1.929</td>
<td>0.267</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>Emotional effect</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
<td>1.357</td>
<td>0.929</td>
<td>0.175</td>
</tr>
</tbody>
</table>

In addition, a lot of them are suffer the problem of bone loss, where an average of this problem is (1.929), the standard deviation (0.267), and the level of significance less than (0.05).

As for the aesthetic problems (poor appearance) and negative emotional effects (psychological effects) there are no statistically significant prove the occurrence of
this problems between these patients, because the level of significance of these problems was higher than (0.05).

Because the patients with complete teeth less do not have remaining teeth, considered their answers about the problems of drifting teeth and over-eruption are (NO).

**The Summary of T-Test Results**

The patients with complete teeth loss and the patients with missing both the anterior and posterior teeth are most groups that clearly suffer problems among patients who involved in the study.

While those who loss posterior teeth only are less group suffer health problems due to loss of teeth among all of them.

**The results of Pearson Correlation**

The following results shows the general relationship between tooth loss and problems of oral health on the one hand, and then shows the relationship between tooth loss and every problem separately.

The strongest relationship was between tooth loss and temporomandibular disorder (TMD) (0.813). It is a strong positive correlation, and the level of significance (0.000), which is less than (0.05). Whenever increasing the teeth loss, increasing problem of TMD.

Then followed that, the relationship between tooth loss and low chewing ability. It is also a positive and strong correlation (0.730) and was indicative of the level of significance (0.000). Whichever, with increase of teeth loss, the problem of low mastication increasing.

As for the relationship between tooth loss and speech distortion was positive medium-strength, the level of significance reached (0.002). Whichever, with increase of teeth loss, the distortion of speech well increasing.

The relationship between tooth loss and aesthetic problems (poor appearance) was positive slight-strength correlation (1.261), the level of significance less than (0.05). Whichever, with increase of teeth loss, the aesthetic problems will be increasing.

As for the problem of drifting teeth, showed a negative low correlation (reverse relationship) the level of significance was (-0.037). With increase of teeth loss, will decreasing the drifted teeth.

As for the problems of the negative emotional effects and bone loss (resorption) are showed positive correlation low strength (weak), but indicative of the level of significance higher than (0.05), that correlation is not significance in both problems.

While, the overeruption of unopposed teeth is showed a negative correlation (reverse relationship), but the significant is higher than (0.05). Therefore, the correlation is not significance.

The general relationship between tooth loss and the oral health problems (all previous problems), the correlation value was (0.614), and the level of significance was (0.000), which is less than (0.05). It is a positive and strong correlation, whichever, with increasing of teeth loss, the oral health problems will be increasing.

**Discussion**

Studies concerning the epidemiology in dentistry have showed that dental caries and periodontal diseases are the most prevalent pathologies that affect the oral cavity. Previous studies performed by American researchers had suggested that dental caries was the main reason for teeth extraction, and other studies accomplished in New Zealand, Sweden, and even in Brazil confirmed that caries may lead to tooth mortality.12-13.

The association between positional changes and tooth loss.

- Temporomandibular disorder (TMD)

The TMD has been very clear in patients with complete teeth loss, because without teeth, the occlusion plane changed in these patients, therefore disorders such as excessive closures and other problems are exposed.

While, the remaining teeth in patients with partial teeth loss maintained at the occlusion plane in the proper place.

Therefore, the TMD is commonly in complete edentulous patients than partial edentulous patients.

This study concluded that, the correlation is strong
between tooth loss and TMD, unlike that study conducted by Ciancaglini and his colleagues\textsuperscript{14} who have concluded that tooth loss is of little relevance in the etiology of temporomandibular disorders.

- Over-eruption

The overerupted teeth emerged in patients with missing both anterior and posterior teeth only, maybe because this problem needing too many gaps and teeth without opposite in the jaws. The relationship between tooth loss and this problem was negative, because with increase of teeth loss will not be teeth there to occur overeruption on it after that.

- Drifting teeth

The drifting teeth common between patients with loss anterior and posterior teeth clearly, which also for occurs is required many gaps and remaining teeth in the jaws, so the proportion of occurrence it slight. Like previous problem, the correlation between tooth loss and this problem was negative, because with increase of teeth loss will not be teeth there to occur overeruption on it after that.

THE ASSOCIATION BETWEEN FUNCTIONAL CHANGES AND TOOTH LOSS.

- Low chewing ability

The patients who suffered of low mastication on extensively were patients with complete teeth loss, as a known without teeth the chewing ability is absent.

The partial teeth loss patients who suffered of this problem were very simple; because good chewing ability is assured even with remain just (20 well-distributed teeth). Similarly, (Sarita et al., 2003)\textsuperscript{15} he has found these previous results.

- Poor appearance

The aesthetic problem due to tooth loss is clearly in patients who have lost their anterior teeth. These patients have complained about their appearance after the loss of these teeth, because the anterior teeth visible and complementary to the beauty of the face.

Because this problem is restricted by spatial conditions (place of the missing tooth), thus not experienced by all patients with tooth loss. Therefore. The correlation was positive but weak.

- Speech distortion

The patients with missing anterior teeth and with complete teeth loss are suffering from this problem significantly. Because the anterior teeth have an important role in the process of speech and produce pure sound.

Thus, the loss of the anterior or all teeth, the pronunciation and aesthetic would affected.

BONE LOSS (RESORPTION)

The majority of patients’ categories had loss (resorption) in their alveolar bone clearly. Because after tooth loss the bone begins to resorb immediately.

The bone loss among patients with missing posterior teeth were somewhat low. Because the bone in the posterior wider than anywhere, so it needs longer time to be absorbed.

On reverse a study by (Bhaskar)\textsuperscript{2} who concluded that, bone loss is more pronounced posteriorly than anteriorly.

THE NEGATIVE EMOTIONAL EFFECTS OF TOOTH LOSS

The negative emotional effects due to tooth loss are not frequently occurring among patients, because with passage of time getting used to it and accept the event, after it was initially difficult admission.

Moreover, a lot of them not care about teeth loss and did not lend attention to this issue originally. Therefore, the correlation was not significant.

THE ASSOCIATION BETWEEN TOOTH LOSS AND ORAL HEALTH PROBLEMS

Most of previous problems had shown high average occurrence on T-Test and strong relationship on Pearson correlation. Therefore, the general relationship between tooth loss and the oral health problems was positive and strong.
Conclusions

This study demonstrated that tooth loss causes several oral health problems. There is a statistically significant relationship between tooth loss and oral health problems, where the strong value of this relationship was (0.614), and the significance was less than (0.05).

Recommendations

Based on the findings of the study, the following recommendations are made:

· Dental personnel should make an effort to identify individuals with risk of tooth loss on oral health in order to prevent tooth loss.

· The patients with tooth loss should replace their missing teeth to restore function and protect the remaining teeth.

· Improvement of dental laboratories to provide quality replacement of missing teeth at affordable costs.

· Further long-term multicenter studies to evaluate the consequence of tooth loss and assist in giving a more accurate projection of the patients’ needs across the nation is mandatory.

Ethical Clearance: Ethical approval obtained from local Ethic Committee of Sebha University (South Libya).

Source of Funding: self

Conflict Interest: Nil

References


Relationship between inflammatory Response and Pathology of *Listeria monocytogenes*

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²Ass.Prof., Biotechnology College, Al Qadysia University, ³ Samawa Technical Institute, Al Furat Al Awsat Technical University

**Abstract**

*Listeria monocytogenes* is a feebly pathogenic, Gram-positive bacterium and ready to develop additionally at the temperature of 4°C. A man frequently gets influenced by expending tainted water and food. 150 aborted placentas were achieved from women and cultured directly, while other were fixed in formalin buffer to study the effect of CD 45 and CD74 in pathogenesis of *Listeria monocytogenes* associated with placentitis in aborted women. Results showed six placental samples from out 50 were positive for listeria culture, were exhibited high expression of CD 45 and CD74 aborted placenta infected with *Listeria monocytogenes* as a compared to uninfected placenta. This study concluded that a relationship between inflammatory response and pathology of *L. monocytogenes* in placental tissue that were collected from aborted women in Al-Muthana City.

**Keywords:** *Listeria monocytogenes*, aborted women, CD45, CD74.

**Introduction**

Listeriosis is a typical foodborne ailment created by *Listeria monocytogenes*. This bacterium is pervasive and found in all aspects of climate including soil, water and rotting vegetation. Huge aspect of the different instances of listeriosis are brought about by taking of the living being in nourishments [¹]. Sickness as a rule happens in very much characterized high danger gatherings, including pregnant ladies, children and immunocompromised grown-ups, however may incidentally happen in people who have no inclining fundamental condition [²].

As indicated by the reports of CDC, pregnant ladies are normally around multiple times more delicate to listeriosis than sound grown-ups [³]. *Listeria* is Gram-positive bacterium of the slash stick structure, motile and is to be found in water and earth. It is truly proficient for endurance since it develops at the temperature under 3°C and all to around 4°C, and beat likewise the unsafe impact of cooling drying and warming [⁴]. Because of that, it tends to be increased in food, which is kept in cooler. Creatures can be additionally transporters of listeria so by devouring of meat, meat items and milk of the tainted creatures, can be influenced likewise the person. Vegetables, aside from carrot and tomato, likewise can be defiled by this bacterium from earth of compost [⁵]. The acquired creature listeria by food can do the attack onto the gastrointestinal epithelial over the unblemished gastrointestinal parcel. Listeria after that being phagocytes by macrophage, monocytes, or granulocytes. The pathogenesis of this bacterium relies upon the property of *Listeria* for endurance and propagation in the host phagocytes. As an intercellular microorganism that balanced out in the lymph tissue goes into circulation system, hepatic tissue and different organs [⁶]. This study was aimed to isolation and detection *L. monocytogenes* from placental specimens that collected from aborted women and to find out the relationship between inflammatory response and pathology of *L. monocytogenes*.

**Materials and Methods**

**2.1. Patients and Sample Collection**

This study was conducted in Al-Smawa hospital of Al-Muthana City, during the period of January 2020 to April. One hundred and fifty aborted women were secondhand in this study. Placental specimens were achieved from aborted distributed into two slices, one slice cultured directly on 7% sheep blood agar (Difico, USA), Listeria selective medium and Eosin
Methylene Blue (EMB) agar (Himedia, India). The cultures were incubated at 37 C for 3 days, aerobically. Other slice of placenta tissue were stable in 10% buffered formalin, managed usually, and stained with Immunohistochemistry as” charity by Mao et al., [7].

2.2. Statistical methods

Percentages were used as a statistical method to express the results, and the probability (p. value) value was calculated for a significant level at (0.001).

Results

Six placental samples from out 50 were positive for listeria culture and yielded practically unadulterated development of β-haemolytic settlements on the blood agar following 24 hours of brooding. The states of microorganisms were showed up little, approximately 1-2 mm in breadth, round, smooth and whitish-dark. On the Listeria specific agar, the states had comparative attributes and were 3-4 mm in size. Development was not seen on EMB agar. Immunohistochemical (IHC) analysis of placental samples exhibited Positive placental staining for CD 45 and CD 74 (Table 1, 2 and Figure 1).

Table 2. Detection of Actuality of CD45 in tissue samples by using Immunohistochemical assay

<table>
<thead>
<tr>
<th>Notch</th>
<th>L. monocytogenes + ve</th>
<th>L. monocytogenes -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>16.66</td>
</tr>
<tr>
<td></td>
<td>*100%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>16.66</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>83.33</td>
</tr>
</tbody>
</table>

*Significant (p ≤ 0.01)
From results showed only six cases were positive for *L. monocytogenes*, the culture characteristic of *Listeria* same with that reported by [8]. Listeria causes necroperulant inflammation; sever placentitis, infiltration of inflammatory cells, hemorrhage and accumulation of amorphous material. Immunohistochemical assays showed high expression of inflammatory CD (CD45 and CD74) and. Chemokines participate in the progression of the inflammatory process by altering the original by changing the native T-helper 0 response to a T-helper 1, T-helper 2, or a mixed T-helper 1 and T-helper 2 response [9, 10]. In the present study, IFN-γ-specific staining the Listeria invigorates Lymphocyte interceded resistance, which, under the impact of cytokines, pulls in macrophages that produce provocative granulomata where microorganisms are obliterated.

Memory Lymphocytes give a gained protection from Listeria disease, and this may clarify why listeriosis is connected with harm, immunosuppressive treatment, Helps, pregnancy and the youngster. This can likewise represent the perception that neutropenia and issues of supplement or immunoglobulin blend are not related with extreme predominance of the infection [11, 12]. Aside from the resistant status of the host, different elements, which impact whether obstructive illness happens incorporate the destructiveness of the contaminating strain and the size of the inoculum [13, 14]. The infective portion is obscure trophoblast cells, was found fundamentally contrast between the tainted and uninfected proposed that the epithelial CD45 recoloring presumably reflects receptor-bound CD45, since this cytokine is delivered distinctly by Immune system microorganisms and NK cells. Besides, it was found in biopsy tests with positive MNCs, proposing a nearby capture of the cytokine in listeria contaminated cells *L. monocytogenes* disease is related with expanded CD74 creation in humoral resistance and enactment of antigen introducing cells [15]

**Conclusions**

This study concluded that a relationship between inflammatory response and pathology of *L. monocytogenes* in placental tissue that were collected from aborted women in Al-Muthana City.

**Ethical Clearance** taken from department committee

**Conflict of Interest** Nil

**Source of funding** self
References


Epidemiological Study of Burn Deaths at a Tertiary Care Centre in Mumbai

Babaso Kalel1, Pawan Sabale2, Shailesh Mohite3

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Abstract

Death due to burns is an important cause of unnatural death commonly encountered in Medico-legal Practice. This prospective study was conducted on 100 cases of burn deaths caused by flames, hot liquids and flash of electricity. This study was conducted to know the demographic profiles, common risk factors, causes and manners of death in fatal cases of burns. Most of the cases were in the age group of 21 to 50 years with a peak incidence in 21-30 years age group, with female predominance. In the study, maximum cases were due to kerosene stove blast leading to accidental burns, followed by pouring of kerosene as a source of burn.

Key words: Burn, autopsy, Kerosene Stove Blast, Accidental Burn

Introduction

Death due to burns is one of the most important public health problems faced by all nations today. Burns represent an extremely stressful experience for both the burn victims as well as their families. Death due to burning is an important cause of unnatural death commonly encountered in Medico-legal Practice.

In different communities the aetiological factor of burn injuries varies considerably, hence a careful analysis of the epidemiological factors in every community is needed before the planning and implementation of a sound prevention program. This study was conducted to know the demographic profiles, common risk factors, causes and manners of death in fatal cases of burns.

Materials and Methods

This prospective observational study was carried out on persons who died due to burns and were brought for medicolegal post mortem examination at mortuary of department of Forensic Medicine of a tertiary care centre in Mumbai, during the period of one year and three months i.e. from 1st September 2016 to 30 November 2017.

This prospective study was conducted on 100 cases of burn deaths caused by flames, hot liquids and flash of electricity, after approval from the ethics committee for academic research project.

Primary data, in each case, was collected from inquest report, accidental death report and indoor paper records. The percentage of burn injury was recorded by sketch diagram, photographs for easy understanding and interpretation. The percentage of burns was calculated on the basis of Wallace’s rule of nine for adults and Lund and Browder’s chart for children.

Results and Discussion

There were 992 medico-legal post-mortem examinations conducted at the Department of Forensic Medicine and Toxicology attached to a tertiary care...
centre in Mumbai during the study period. Out of which, 100 (10.28%) cases were of death due to burns. In the present study 100 (10.28 %) cases of death due to burns were included.

Table 1- Age & Sex wise distribution of deceased

<table>
<thead>
<tr>
<th>Age category</th>
<th>Male</th>
<th>Female</th>
<th>Total No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 10</td>
<td>02</td>
<td>02</td>
<td>04</td>
</tr>
<tr>
<td>11 to 20</td>
<td>03</td>
<td>06</td>
<td>09</td>
</tr>
<tr>
<td>21 to 30</td>
<td>14</td>
<td>22</td>
<td>36</td>
</tr>
<tr>
<td>31 to 40</td>
<td>09</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>41 to 50</td>
<td>06</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>51 to 60</td>
<td>01</td>
<td>03</td>
<td>04</td>
</tr>
<tr>
<td>61 to 70</td>
<td>02</td>
<td>06</td>
<td>08</td>
</tr>
<tr>
<td>71 to 80</td>
<td>00</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>81 to 90</td>
<td>01</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>91 to 100</td>
<td>01</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

Most of the cases (72%) were in the age group of 21 to 50 years with the peak incidence in 21-30 years age group, with female predominance. This is similar to the observations of Chawla R et al\(^1\) and Dasari H et al\(^2\). It shows proneness of the young population to hazards of fire. This is the most active group of people where the females are more concerned with the kitchen hence more chances of sustaining accidental burns. Females outnumbered males in all age groups except in 0-10 and 71-80 age groups. House wives were involved in 45% cases followed by workers in 21% cases, others victims were students, vegetable venders, car washers, garment painting, and farmers. This is consistent with a study done by Deshpande et al.\(^3\) This may be due to the fact that in children and elderly both sexes are equally and occasionally exposed to fire.

Table 2 - Marital Status of deceased

<table>
<thead>
<tr>
<th>Marital status</th>
<th>No of cases</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>82</td>
<td>30</td>
<td>52</td>
</tr>
<tr>
<td>Unmarried</td>
<td>07</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Not applicable</td>
<td>11</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>39</td>
<td>61</td>
</tr>
</tbody>
</table>
The data shows that maximum incidence of burn deaths occurred in married people i.e. 82 as compared to unmarried people which is only in 07 cases and 11 cases were below the legal age of marriage. Out of married 82 people, there were 52 females and 30 were males, it shows female predominance. In this study, 36 married females out of total 100 victims died within seven years of marriage. Out of these 36 married females only in one case there was allegation of demand for dowry.

This finding is consistent with Dasari H et al\(^2\) and Tasgaonkar et al\(^4\). This is an indicator of social problems among married people, especially females, who have all household responsibilities. Also younger and newly married females may become the victim of dowry demand and domestic violence.

Maximum 77 (77 %) incidences occurred inside the house i.e. in close place (kitchen, bedroom, living room), followed by whereas 18 (18 %) were reported outside the house i.e. in open space. 05 (05%) incidences were reported at the workplace. This is because the housewives working in the kitchen are more prone to hazards of fire. Most of the suicidal victims prefer closed spaces like a living room.

<table>
<thead>
<tr>
<th>Percentage of burn</th>
<th>Survival period after incidence</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Less than 24 hours</td>
<td>1 to 7 days</td>
</tr>
<tr>
<td>0 to 20</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>21 to 40</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>41 to 60</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>61 to 80</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>81 to 100</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>No of cases</td>
<td>12</td>
<td>50</td>
</tr>
</tbody>
</table>

More deaths due to burns 40 (40%) occurred in those who sustained 41 to 60 % of burns, 26 (26%) deaths occurred who sustained 61 to 80 % of burns and 17 (17%) deaths occurred in 81 to 100 % burns. Fewer deaths caused due to burns occurred in 9cases who had sustained 0 to 20 % burns, 8 deaths occurred in those who sustained 21 to 40 % burns. Maximum 50 (50%) people survived for one to seven days after the incidence of burn followed by 23 (23%) who survived for eight to fourteen days and 12 (12%) people survived for less than twenty-four hours. These findings suggest that the more the percentage of burn, less is the survival period and vice versa.
Table 4 - Distribution of source of burn of deceased

<table>
<thead>
<tr>
<th>Source of burn</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerosene stove blast</td>
<td>35</td>
</tr>
<tr>
<td>Pouring Kerosene</td>
<td>22</td>
</tr>
<tr>
<td>Electricity flash burn</td>
<td>09</td>
</tr>
<tr>
<td>Fire lamp</td>
<td>07</td>
</tr>
<tr>
<td>Hot liquid</td>
<td>05</td>
</tr>
<tr>
<td>LPG Gas</td>
<td>05</td>
</tr>
<tr>
<td>House Fire</td>
<td>04</td>
</tr>
<tr>
<td>Fire Wood</td>
<td>04</td>
</tr>
<tr>
<td>Boiler Explosion</td>
<td>03</td>
</tr>
<tr>
<td>Inverter Battery Blast</td>
<td>02</td>
</tr>
<tr>
<td>Hot Milk</td>
<td>02</td>
</tr>
<tr>
<td>Fire at Warehouse</td>
<td>01</td>
</tr>
<tr>
<td>Cracker Blast</td>
<td>01</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Kerosene stove blast was leading source of accidental burns in 35 cases, followed by pouring of kerosene as a source of burn in 22 cases. This finding is consistent with Chawla et al.\textsuperscript{1}

Maximum deaths due to burns i.e. 91 (91%) occurred were dermo-epidermal type of burns followed by 08 deaths (08%) due to epidermal burns and 01 death (01%) due to deep burns. Out of 100 burn deaths, 97 victims were hospitalized and 3 were brought dead. The nature of burn injuries in all 100 victims was antemortem.

In all types of thermal burns, the upper half of the body was observed to be involved more than the lower half of the body. Similar findings were also observed in a study done by Deshpande et al\textsuperscript{3}, who observed that anterior and posterior trunk were most commonly affected followed by lower extremity. In a study done by Mustafa F\textsuperscript{5}, maximum involvement of upper limbs (66.8%) was observed followed by lower limbs (49.1%).
Table 5 - Distribution of immediate cause of death

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia &amp; septicemia</td>
<td>37</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>19</td>
</tr>
<tr>
<td>Septicemia</td>
<td>20</td>
</tr>
<tr>
<td>Neurogenic Shock</td>
<td>11</td>
</tr>
<tr>
<td>Hypovolemic shock</td>
<td>06</td>
</tr>
<tr>
<td>Others</td>
<td>07</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

It is observed that in maximum 37 cases (37%) the immediate cause of death was pneumonia and septicemia. In 19 cases (19%) the cause of death was pneumonia, 20 cases (20%) it was septicemia, 6 cases died due to hypovolemic shock and 11 cases died due to neurogenic shock. Out of 11 cases, in 08 cases the cause of death was neurogenic shock due to electrocution and in three cases the cause of death was neurogenic shock due to 95 to 100 % burns. The other causes include acute renal failure, acute respiratory failure, shock and suffocation and head injury secondary to electrocution.

Chawla R et al\(^1\) observed 22 % cases died due to primary shock, 10 % died due to oligemic shock and 56% died due to Septicemic shock and 12 % died due to injuries. Death within the first 72 hours is due to loss of fluid leading to hypovolemic shock. As the survival period and hospital stay increases, chances of infection increases leading to septicemia.

On the contrary, Nath et al\(^6\) observed shock as the cause of majority deaths i.e. 65.74 %, followed by septicemia 28.7 % cases. In a study done by Tasgaonkar et al\(^4\), observed 3.38 % deaths due to Neurogenic shock, 36.38 % cases due to oligemic shock, 29.54 % cases due to Septicemic shock and 30.46 % cases due to acute tubular necrosis and complications of septicemia.

The manner of death in 78% cases was accidental, 18% cases had committed suicide and 04 % were homicidal in nature. This finding is consistent with Tasgaonkar et al\(^4\) and Buchade et al.\(^7\) This might be due to the fact that maximum victims in the present study were housewives who were more prone to accidental contact to fire while working in the kitchen, particularly in small and crowded houses. This is not consistent with a study of Nath et al\(^6\) which shows maximum burn deaths i.e. 177 (81.94 %) which were suicidal followed by 35 (16.20 %) homicidal and least cases 4 (1.85 %) were accidental.

**Conclusion**

Females aged between 21 - 40 years are more susceptible to burn injuries. Maximum numbers of victims were housewives and died due to accidental burns. However accidental burn deaths are preventable through a combination of prevention strategies and improvements in the care of people affected by burns. The cases of burns with alleged dowry demand are very low in this study. This may be due to mass and social media in creating awareness among the society regarding laws dealing with dowry death or non-reporting of the dowry issue at the time of post-mortem examination.

Kerosene stove blast was observed to be the most common source of accidental burns. These stoves
provide a very cheap alternative to electric or gas stoves, especially in low socio-economic groups. The accidents are usually due to the fact that the victims do not follow the instructions and do not observe the necessary precautions.

**Conflict of Interest** - None

**Source of Funding** - None

**References**


Assessment of Occupational Stress in Medical Doctors Working in Tertiary Care Hospital

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Abstract

Background: Nowadays, stress has commonly been assessed especially in medical doctors. The reason for the same is that medical doctors have to play high competitive roles and in turn have so many responsibilities keeping in mind the holistic care to patients.

Aim/objective: To assess occupational stress in medical doctors working in tertiary care hospital of Bhavnagar, Gujarat.

Materials and Methods: This cross-sectional study was conducted during September 2017 to December 2017 after prior approval of institutional ethics committee. Total 75, both male and female medical doctors were enrolled as study participants after written informed consent and based on inclusion criteria. Along with case record performa, self-administered pre-tested questionnaires having stress scale namely Professional Stress Scale (PSS) and Perceived Stress Scale 10 items (PSS-10) were given to all participants as a study tool. The data of scores received on scales was and entered in Microsoft excel and the same was analyzed by using GraphPad Prism 5 software for various proportions, associations and frequencies. The associations were considered significant if the p value is less than 0.05.

Results: Out of 75, total 50 participants filled and submitted the questionnaires. The mean age of participants was 31.96 years. As per the stress scales used, moderate stress was observed among medical doctors irrespective of sex. The commonest symptom observed among stressed doctors was sleep disturbances (insomnia).

Conclusion: Moderate to mild stress was found among medical doctors. Stress in medical doctors is never underestimated irrespective of sex. Insomnia is the commonest symptom observed among stressed doctors.

Keywords: Occupational Stress, Medical Doctor, Insomnia

Introduction

Stress is a widely spread and considered very common phenomenon which affects not only the individuals but also organizations and societies. Due to globalization and increased complexities of work and demands, stress has now become a prominent and permeating problem to everyone. Cobb and French (1975) defined occupational stress as “any characteristics of job environment which poses a threat to the individual”. Copper and Marshall (1976) have expressed that “by occupational stress is meant negative environmental factors or stressors associated with a particular job”.¹ Stress can be generally defined as unwanted, improper or overemphasized response to a situation. Person’s anxiousness to a particular situation
can be positive but stress is always being considered as negative which in turn affects physical and mental well being of a person. Stress in medical practice has always been a topic of interest owing to its adverse outcomes. The reason for the same is that medical services involve not only treatment of patients but also a holistic care. Therefore, any negligence or mistakes in patient’s care could be very costly and irreversible. With such huge responsibilities, it is always expected from a medical doctor that he/she should be free from any kind of worries, tension or mental pressure rather he/she should have perfect state of mind. But in reality, medical doctors are likely to get stress due to complex work pattern and lot of expectations from society.\(^2\) Moreover, the modern medical work environment is very compound and to which different doctors respond to it differently. Some are finding it encouraging and fruitful, whereas others may feel anxious, stress and annoyed from the heavy workload. The medical work practices are constantly being evolved by incorporating newer skills and techniques in patient’s care due to advances in medical knowledge. This in turn leads to changes in doctor’s work pattern due to professional and career development competitions. The heavy workload and the health risks faced by the doctors put them under lot of stress. Doctors have higher rates of suicide, psychiatric illness and possibly alcohol and drug misuse than the general population.\(^3,4\) Stress has a direct impact in employee’s health and consequently impacts work performance. It also leads to psychosomatic disorders such as asthma, diabetes, backpain, hypertension, anxiety, depression and arthritis.\(^5\) One of the leading causes of disability by the year 2020 have been identified by WHO is stress related disorders.\(^6\) Studies which measured stressful life events found that approximately 90% of suicide attempters reported negative life events\(^7\) and about 35% experienced stressful life events in the previous 6 months.\(^8\) with the passage of time, greater emphasis is given to assess stress in medical doctors and to find out its sources. The major adverse outcome of having persistent work related stress in doctors is poor quality in patient care which in turn affects services to mankind.\(^9,10\) Therefore, this study was undertaken to assess stress among medical doctors working in tertiary care hospital in Bhavnagar, Gujarat.

### Materials and Methods

Study design of present research was cross-sectional. The data were collected from the medical doctors working in tertiary care hospital in Bhavnagar, Gujarat. The study was approved by ethics committee. Participants were chosen irrespective of their designation, gender and qualification. Present study was carried out from September 2017 to December 2017. A total 75 doctors were approached to participate in the present research study. They were selected by random sampling method from the employee list provided by Hospital HR department. Their enrollment was based fulfilling the inclusion criteria mentioned below:

1. He/she willing to participate in the study
2. He/she has more than 1 year of working experience in same profession
3. He/she is working in any of the shifts in Hospital
4. He/she has no any known disease

Informed written consent was obtained from all study participants before starting the data collection and they were assured for confidentiality of their information. In every step of this survey, anonymity of study participants was maintained. The study was entirely based on the self-administered and pre-validated questionnaire for the assessment of stress levels. The questionnaire had two parts: **Part 1** – Demographic details including work experience, working hours, work scheduling, breaks and number of dependents and **Part 2** – Included two stress scales, Professional Life Stress Scale\(^11\) and Perceived Stress Scale 10 Items.\(^12\) The Professional Life Stress Scale, scores can range from 0 to 60 and classified into 4 different classes depending on the obtained scores. Scores between 0 and 15 indicates no stress, 15 to 30 indicate moderate stress, 30 to 45 indicate stress needing remedial action and 45 to 60 indicates stress as a major problem needing intervention without delay. Perceived Stress Scale 10 item measures the degree to which the situations in one’s life are appraised as stressful. Scores can range from 0 to 40 with higher scores indicating greater association. The responses of questionnaire were entered in Microsoft Excel and analyzed using GraphPad Prism 5 software for various proportions, associations and frequencies. The associations were considered significant if the p value is less than 0.05.
Results/ Observations

In this study the response rate for the self administered questionnaire among medical doctors was 50 (66%) means 50 participants have submitted filled questionnaires out of total 75 enrolled.

Demographic details: Among the 50 respondents, 50% were male and 50% were female. Most of the doctors (both among male and females) were in the age group of 20-30 years, 14 (28%) and 16 (32%) respectively. The mean age of the respondents was 31.96 years (Table.2). The number of dependents varied between nil to 5 and the mean number of dependents was 1.36. On an average the doctors worked for 11.08 hours per day. Majority of the doctors were eligible to take two breaks of 15 minutes each for tea/coffee and one break of 30 minutes for lunch. Most of the doctors travelled 5 minutes to 30 minutes to reach their place of work. Majority, 38 (76%) of the respondents had a work experience of 10 years or lesser (Table.3).

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤25</td>
<td>4 (8)</td>
<td>10 (20)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>26-30</td>
<td>10 (20)</td>
<td>6 (12)</td>
<td>16 (32)</td>
</tr>
<tr>
<td>31-35</td>
<td>5 (10)</td>
<td>1 (2)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>≥36</td>
<td>6 (12)</td>
<td>8 (16)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>Total</td>
<td>25 (50)</td>
<td>25 (50)</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Work experience (years)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 5</td>
<td>26 (52)</td>
</tr>
<tr>
<td>6 to 10</td>
<td>12 (24)</td>
</tr>
<tr>
<td>≥11</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

Stress scores:

Scores of the Perceived Stress Scale:

00 – 10: 11 (22%)
11 – 20: 19 (38%)
21 – 30: 20 (40%)
31 – 40: 0 (0%)

Analyzing the perceived stress scale, most of the doctors (40%) scores ranged 21-30 followed by 11-20 and 00-10. The mean score was 16.66.

Scores of the Professional Life Stress Test:

00 – 15: 19 (38%)
16 – 30: 29 (58%)
31 – 45: 2 (4%)
According to the professional life stress test, 2 doctors (4%) were stressed, needing immediate remedial intervention. Out of this two, both were male doctors. 29 (58%) were moderately stressed and 19 (38%) had no stress. Among the 29 with moderate stress, 15 (51%) were males which underlines that stress is more among men when compared to women and this difference was not significant.

There was no significant association between any of the study variables like gender, total work experience, number of dependents, total work hours, break time and the stress levels according to both perceived stress scale and professional life stress test.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestion problem and poor appetite</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Sleep problems</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Excessive sweating</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Tiredness and hopelessness</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Irritation</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Inability to unwind</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Lack of self-confidence</td>
<td>4 (8)</td>
</tr>
<tr>
<td>More workload than normally managed</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Poor decision making</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Nervousness</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Sadness with weeping</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

All the 50 doctors had at least one symptom of stress and the symptom of stress which troubled the most was considered and depicted in the table 4. Problems associated with sleep were the most common stress symptom among the participants with most of them having difficulty going to sleep. Digestion problems and poor appetite, tiredness and hopelessness, excessive sweating, inability to unwind and lack of self-confidence were the other symptoms of stress among the respondents.

Discussion

Professional stress leads to adverse effects which belong to both physical and emotional. It occurs when efforts and dedication at workplace did not match with ability, resources and financial expectations of the person who is employed. Special emphasis was given to professional stress as it has harmful effects on human health and can disturb social and personal association. It has been observed in India that doctors who are working in Government setup were provided least
facilities and resources. Moreover, they have issues with regards to salary as compared to workload. Hence, medical profession is considered as one of the heavily work occupied profession with minimal incentives for healthcare providers. Therefore, present efforts targeted doctors acquainted with Government hospital, Bhavnagar on the basis of the stress severities. Majority of the participants were belong to age less than 30 years which also suggested that they were eager to participate in the study and also knew that they might have some kind of stress. We enrolled both male and female doctors the ratio of which is 1:1. Our findings matched with the studies done by Passey et al\(^\text{15}\) and Azmi et al.\(^\text{10}\) In the demographic details, the male doctors have more numbers of dependents than females which may enhance the existing level of stress in them. Average working hours of doctors was 11.08 hours which is somewhat higher than the average population. Moreover, these hours are full of attention and determination in patients’ care. As per the stress scales results, this study showed moderate to mild stress among medical doctors. Our findings were also supported by the various studies,\(^\text{3, 10, 15, 16}\) PSS-10 scores for majority of doctors in this study was between 21-30 which must be monitored and if the scores show an increasing trend, then it suggests the need for intervention. The professional life stress test scores suggest that few employees are stressed and the need to adopt stress management techniques. Even this trend has to be monitored and intervention started when needed. More importantly, the company can adopt stress management trainings (SMT) even short duration SMT would be beneficial both for the doctors and the hospital. Among the various symptoms of stress, insomnia was the most common among doctors which suggest that if sleep disturbances are there it should be taken into consideration immediately.

**Limitations of the Study**

1. The study had a small sample size which did not give proper idea about actual stress levels among medical doctors.
2. The participants were reluctant to give proper response in terms of filling questionnaire and submit the same. Out of total 75 questionnaire distributed, only 60 filled questionnaires were received. Among them 10 questionnaires were incomplete, so they were rejected and rest 50 completely filled questionnaires were considered for the study.

**Conclusion**

Increased stress levels are a major problem affecting the doctors working in tertiary Government hospital. Moderate to mild stress was found in medical doctors. Sleep problem is the most common symptom during stress. Stress at workplace has a major adverse implication both at individual as well as the organizational.

**Conflict of Interest:** None

**Source of Funding:** Self

**References**

Pre-Analytical Errors in Clinical Diagnostic Laboratory: A Crucial Step to Look for Accuracy and Reliability

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Abstract

Clinical Diagnostic Laboratory (CDL) plays a very important role in diagnosis and treatment of diseases. Total testing process (TTP) of laboratory includes pre-analytical, analytical and post-analytical phases. To have an accurate and reliable results, detection and prevention of errors is must from all these phases. Chances of errors are more in pre-analytical phase as compared other two phases. Though standards have been made to control error occurrence in pre-analytical phase, errors still noticed in this phase. The reason is all the steps involved in this phase are dependent on humans and thus it is out of control of laboratory. Therefore it is necessary to generate proper guidelines or manual to minimize errors in pre-analytical phase. This is also an important step to achieve Total Quality Control (TQC). We have tried in this review to summarize important pre-analytical errors, their occurrence at various stages, prevalence and preventive aspects.

Keywords: Clinical Diagnostic Laboratory, total testing process, pre-analytical errors

Introduction¹⁻⁵

In current era, disease diagnosis is mostly dependent on accurate and reliable laboratory results. Thus, the role of laboratory became very crucial to ensure the best possible results outcome from its analysis process. Nowadays performance of laboratory drastically improved due to advancement in technology like automation, sample collection, its transport and reports delivery. But it is very difficult, not impossible, to achieve 100% accuracy and reliability in laboratory performance. As it is rightly said that errors are bound to occur in any endeavor, analytical process of lab also faces errors. These errors are classified as pre-analytical (test order to receipt of sample in lab), analytical (sample processing and analysis) and post-analytical (report generation to its dispatch). Out of these three, pre-analytical errors are the most commonly occurred errors in total testing process (TTP) of laboratory. Study showed 46 × 71% errors encountered during TTP were belonging to pre-analytical phase. Hence, this phase must be monitored carefully to avoid such errors. Pre-pre-analytic and actual pre-analytic are two areas of the pre-analytical phase. Selection of Tests to be done, identification of patient for sample collection, preparation and its handling are part of the pre-pre-analytical process. Storage of samples and its processing in lab i.e. pipetting and centrifugation are part of actual pre-analytical process. All the errors occurring before the sample is processed for analysis in laboratory are considered as pre-analytical errors. These include improper test request, fault in sample collection, transportation errors and errors in request forms filling. These all errors are under control of human as it is carried out manually, therefore laboratory have no any control over it. But still all the responsibility lies on the laboratory as finally report is dispatched by lab. Hence,
laboratory must ensure error-free pre-analytical phase which is a crucial step in quality control (QC) whereby lab maintains its quality.

**Pre-Analytical Errors**

Pre-analytical errors encompass all the administrative and functional errors that occur prior to laboratory analysis of samples. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have developed the range of pre-analytical phase quality markers to underscore pre-analytical errors. The pre-analytical phase error variables are shown in Table-1.

<table>
<thead>
<tr>
<th>Table-1: Variables of pre-analytical phase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient Variables</strong>*</td>
</tr>
<tr>
<td><strong>Collection Variables</strong></td>
</tr>
<tr>
<td><strong>Handling Variables</strong></td>
</tr>
</tbody>
</table>

*Uncontrollable variables

List of commonly occurring pre-analytical errors is as below:

- Patient identification error
- Sample labeling error
- Erroneous blood collection or wrong mixture ratio
- Early clotting
- Collection in wrong vacutainer
- Hemolyzed sample / lipemic sample

- Increased RBCs and decreased plasma volume
- Effect of temperature/ sunlight
- Lack of timely transportation to lab
- Improper handling of specimen

Patient identification is most important first step in blood sample collection. Error in patient identification is very serious in terms of poor outcome of patient care which can be irreversible. Error in identification may be due to heavy workload experienced by staff in hospital.

Table-2 showed pre-analytical errors observed in previously conducted studies.

<table>
<thead>
<tr>
<th>Table-2: Pre-analytical errors observed in various studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Bhavsar M et al11</td>
</tr>
</tbody>
</table>
As per the table-2, it can be concluded that

1. the most common pre-analytical errors observed are:
   - Hemolysed blood samples
   - Clotting blood samples/ tube factor
   - Inappropriate form/ request form filling

2. Pre-analytical errors are observed mostly in indoor patient departments (IPD)

**Prevention**

Pre-analytical errors mostly occurred with the blood specimen followed by urine and other body fluids. The sources of errors with their possible prevention is given in table-3.
<table>
<thead>
<tr>
<th>Phase of pre-analysis</th>
<th>Source of error</th>
<th>Possible prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient identification</td>
<td>Wrong or incomplete Information on test request form</td>
<td>Use Admission register or ward record Patient identification bands</td>
</tr>
<tr>
<td>Patient preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collection after taking food for tests requiring fasting sample</td>
<td>Proper instruction and patient preparation i.e overnight fasting Verify proper preparation before blood collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analytes which can be affected by diurnal variation</td>
<td>Average of the results of two samples drawn at different times of the day</td>
</tr>
<tr>
<td>Test request form</td>
<td>Inappropriate form/ request form filling</td>
<td>Training for proper request form filling Sample filled form can be provided</td>
</tr>
<tr>
<td>Site of blood collection</td>
<td>Wrong site selected</td>
<td>Choosing the correct site Refer blood collection manual</td>
</tr>
<tr>
<td>Site preparation</td>
<td>Contamination with alcohol</td>
<td>After applying alcohol/spirit swab, allow the site of puncture to dry then collect sample Refer blood collection manual</td>
</tr>
<tr>
<td>Tourniquet Application and Time</td>
<td>Applied for longer duration</td>
<td>Recommended time for application is less than one minute Refer blood collection manual</td>
</tr>
<tr>
<td>Proper Venipuncture Technique</td>
<td>Excessive probing and or fishing</td>
<td>Proper training for phlebotomy Refer blood collection manual</td>
</tr>
<tr>
<td>Order of Draw</td>
<td>Incorrect order of draw can lead to potential cross contamination</td>
<td>Order that should be followed: tube for blood culture, citrate tube, serum tube (plain), Heparin, EDTA and fluoride tube Refer blood collection manual</td>
</tr>
<tr>
<td>Sample volume and Tube filling</td>
<td>Erroneous phlebotomy technique and tube mixing</td>
<td>All tubes with additives should be mixed evenly with the correct volume of blood as per the request Refer blood collection manual</td>
</tr>
<tr>
<td>Tube Handling and Specimen Processing</td>
<td>Erroneous sample handling and processing</td>
<td>To follow guidelines mentioned in blood collection manual</td>
</tr>
<tr>
<td>Handling of Blood Specimens in special conditions</td>
<td>Faulty handling</td>
<td>Special training to be given to phlebotomist</td>
</tr>
</tbody>
</table>
For urine specimen, guidelines given by Clinical and Laboratory Standard Institute (CLSI) should be followed.\textsuperscript{21} Cerebrospinal fluid (CSF) specimen also forms an important part of CDL. For the collection of CSF, sterile screw-cap tubes should be used. Preferably clinician or resident doctors should collect CSF. Blood contaminated CSF sample must be discarded. CSF sample should be immediately sent to lab after collection. After receiving in lab, it must be centrifuged first before analysis. For preservation, sample should be stored in small aliquot tube to be filled up to 75\% to avoid adsorption and evaporation.\textsuperscript{22, 23}

Conclusion

The most common errors of pre-analytical phase in clinical laboratory observed are Hemolysed blood samples, mistakes in the filling of tubes or inadequate anticoagulant-blood ratio or tube factor, followed by patient misidentification or improper request form filling. These errors can be prevented by following proper guidelines laid down by various bodies, getting trained in phlebotomy and referring blood collection and other manuals in lab. This forms a very crucial component of laboratory quality. Blood collection manual should address not only patient variables but also specimen variables. Training should be provided regarding pre-analytical manual to all the concerned personnel who are part of health care services. Awareness among clinician/physician regarding such errors and its prevention should be generated which ultimately help in reducing pre-analytical errors.

Search Strategy

We searched Google and PubMed with the terms: “pre-analytical errors”, pre-analytical phase”, and “biochemistry lab” in combination with “quality control”, “total testing process”, “clinical chemistry”, “laboratory”, “analytical phase”, “analysis”, “clinical biochemistry laboratory”. We gave preference to papers published within the past 20 years, but did not exclude some important less recent publications.

Ethical Approval

This study did not warrant institutional review board review as no human subjects were involved.

Source of Funding: Self

Conflict of Interest: None

References


Primary Prevention of COVID 19

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Abstract

Coronavirus disease - 19 is caused by a newly discovered virus called coronavirus. Coronavirus is a spherical, enveloped particle with a single stranded RNA which causes acute mild respiratory illness. The transmission is via airborne droplets. It multiplies in the nasal mucosa causing cell damage and inflammation. COVID 19 has been increasing exponentially as it spreads very fast and in a country like India with a population of 135 crore it is difficult to curtail the spread of the virus. The steps taken to control this spread such as social distancing, nation-wide lockdown were important to restrict the geographical spread of coronavirus. The virus is transmitted by droplets which can travel in air when an infected person or carrier coughs or sneezes. Therefore, primary preventive measures like practicing hygiene with soap and water for at least 60 seconds or using alcohol-based hand rub, social distancing and lockdown were implemented. This article covers the method of hand washing, steps of hand washing and gives information about the do’s and don’ts of alcohol-based hand rubs. It also covers the importance of social distancing and lockdown with the social distancing methods adopted in India.

Key words: Coronavirus, hand washing, prevention, alcohol based sanitizers, masks, mode of transmission, social distancing, N95, respirators, droplet, lockdown, biomedical waste management, disposal, labelled containers.

Introduction

Coronavirus disease - 19 is caused by a newly discovered virus called coronavirus. Coronavirus is a spherical, enveloped particle with a single stranded RNA which causes acute mild respiratory illness. The transmission is via airborne droplets. It multiplies in the nasal mucosa causing cell damage and inflammation. This is manifested as sneezing, cough, cold, nasal obstruction and fever. (1)

The disease spreads by coming in contact with an infected person through droplets and since most people could be asymptomatic carriers the best way to prevent the spread is to be informed about the preventive measures for the society at large. There is no medication or vaccination available for this virus right now. All vaccinations are under trial and therefore won’t be available anytime soon. Primary prevention, that is, aiming to prevent the disease even before it occurs has now become the only means of curtailing the spread of coronavirus.

Method

Published articles, documents were reviewed and compiled together to constitute this article.

Hand Hygiene

Hand hygiene is now considered as one of the most important steps in infection control protocols. With an increasing number of healthcare-associated infections and multi drug resistant pathogens health care practitioners have realised that it is time to go back to the fundamentals of infection prevention by using uncomplicated and cost effective widespread measures like hand hygiene. This has occurred because of scientific evidence backing the fact that simple measures like adopting proper hand washing can on their own reduce the risk of cross transmission of infections in healthcare.

The hands of healthcare workers are colonized with multi drug resistant pathogens which can survive for as long as 150 hours. Around 1000000 skin cells are shed in a day that can contaminate the immediate surrounding of the patient.
Importance

Hand hygiene has become one of the most important and the least costly methods in reducing transmission of infection.

The objects we touch during the day such as door knobs, mobile phones, car keys etc act as fomites i.e. fomites are the inanimate objects that serve as mechanism of transfer of infection between hosts.

There are two types of flora on the hand; transient and resident flora.

Transient flora is present in the superficial cells and the resident flora is present in the deeper layers. These transient microorganisms can be removed easily by hand washing and are the main source of spread of infection in a healthcare facility.

MRSA or methicillin resistant staphylococcus aureus has become one of the most challenging problems faced in the ICU and studies have shown that increase in compliance for hand washing has shown a fall in MRSA rates.

The most important problem faced after knowing the importance of handwashing is knowing when to use a sanitizer and when to wash hands with soap and water.

(2)

Major determining factors should be cost, availability of product, requirements from the product and the presence or absence of visible contamination of hands.

Advantages of using plain soap:
1. Good for removal of soil and proteinaceous material from hands.

Disadvantages of using plain soap:
1. Not bactericidal
2. Can cause dermatitis

Advantages of using alcohol compounds:
1. Faster onset of action
2. Effective against bacteria, enveloped and non enveloped viruses.

Disadvantages of using alcohol compounds:
1. Cannot be used when hands are visibly soiled
2. Flammable
3. Volatile
4. The type of alcohol, concentration, duration of contact and method of application all play a key role in its action.

Wash hands with soap and water when
1. Visibly soiled with blood and other body fluids
2. After using the washroom
3. Prior to and after meals.
4. After blowing one’s nose, coughing or sneezing
5. After providing routine care for someone who needs assistance

Alcohol based hand rub should be used
1. When in direct contact with the patient
2. Before wearing gloves
3. Before inserting self-retaining catheters etc
4. Before and after examining patients

Method of hand washing
Figure 1: Steps of Handwashing (World Health Organisation, 2020)

Method of hand washing:

Before washing hands remove all accessories such as bangles, wrist watches, rings etc.

Following the given steps is important because when we wash hands in a hurry some areas of the palm are neglected such as thumbs, tips of fingers and interdigital clefts.

Wet hands under running water, lather with soap and follow the steps mentioned in the image above. Wash hands under running water. Air dry or use a single use disposable towel to dry the hands.
Even with the on-going attempts to spread awareness about the importance of hand hygiene there are some factors that influence the compliance of health workers to hand hygiene: clinical factors such as working in an ICU, understaffed hospitals, weekdays, camps where there’s no time for the health care professional to practice proper hand hygiene (4,5).

Other factors include lack of guidelines and protocols in institutions, lack of knowledge, when wearing personal protective equipment, forgetfulness, shortage of soap and water supply (6).

Methods to improve hand hygiene:

It takes 21 days to form a habit and that’s why the practice of hand hygiene shouldn’t just be taught in medical colleges, it should be practiced.

With the idea of increasing the clinical exposure of medical students in colleges such practices should be taught then.

Hospital administration should install sinks and maintain adequate supply of soap, sanitizers and disposable towels should be available in every ward, doctors must use hand sanitizer after visiting every patient in the ward, the nursing staff, cleaning staff should be taught the right method. (2)

The most important step in increasing compliance would be to adapt these activities at the peripheral health centres since they are the first and the most commonly visited place by a patient. The staff at a peripheral health centre should be taught the right method and should explain it to the patients as well. Hand hygiene demonstrations should be conducted for them.

Other steps like positive role modelling and the use of performance indicators also improve the adherence to hand hygiene (7).

It should be emphasized that wearing gloves does not eliminate the need for hand hygiene and that contamination may occur during glove removal. Hospitals should also have an infection control team and should strictly follow protocols made by them.

The problems of non-compliance in the Indian scenario are due to factors like overcrowding, limited number of doctors due to high cost of medical education, understaffing, poor infrastructure such as lack of sufficient hand facilities such as sinks, running water and sewage system, lack of awareness and education, ignorance and the fact that most people are losing faith in doctors it’s hard to adapt such practices in our society.

Figure 2 Areas often missed on improper hand washing
Handwashing or the use of alcohol based sanitizer has become a necessity now and is now practiced not only at hospital entrances but also at places of worship, airports, cabs etc. This clubbed with social distancing to reduce the spread of coronavirus.

**Masks**

Considering the route of spread of infection i.e. droplets, the use of masks to curtail the spread of infection becomes evident. The droplets can further form aerosols and travel longer distances and remain in the air for longer periods of time therefore the use of masks at all times is crucial.

There are various types of masks available for people to use but they should be used according to the following factors - profession, places visited, duration of contact with people and risk of development of complications in people who have comorbidities such as HIV/Diabetes, elderly and pregnant females.

Doctors and the entire hospital staff should wear N95 masks. The panic and the fear caused by the coronavirus outbreak has led to a high demand of these N95 masks and therefore guidelines for the use, reuse and extended use of N95 masks has been provided to the health care professionals.

**The Physics behind N95 masks:**

Once an airborne particle touches the fibre in the mask it stays stuck to it and isn’t airborne anymore. This mainly depends on the particle size.

Particles larger than 1 micrometer travel in a straight line due to inertia and because these masks have a lot of these sticky fibres, it’s bound to stick to one of those. This is called “capture by inertial impaction”.

Particles smaller than 0.1 micrometer collide with air molecules and move in a zigzag motion called the Brownian movement. This makes the particle stick to a fibre and is called “capture by diffusion”. Intermediate size particles are difficult to filter because they do not move like the other size particles and therefore can move around the fibre with the air. But N95 masks capture these particles using an electric field. This is called as “capture by electrostatic attraction”. And because it filters 95% of such particles, it is called an N95 mask.

Extended use of the mask means using the same mask for repeated exposure to several patients without removing the respirator in between patients.

Reuse is the use of the same N95 respirator for multiple patients but removing it after each encounter.

Perform hand hygiene before and after touching the respirator. A face shield could be worn over the mask for additional protection.

When to discard the respirators?

1. When used after an aerosol generating procedure.
2. Visibly soiled respirators should be discarded
3. After coming in close contact with an infected patient.

Controlling the spread of respiratory infection using a mask is a well established strategy.

The use of face masks can account for the different levels of prevention i.e primary as well as secondary.

Primary prevention aims at reducing the incidence of disease by elimination of risks for the protection of personal and community health.

Secondary prevention aims at reducing the prevalence by shortening the duration of the disease. This is done by early detection and interventions to minimise disability. This is practiced everywhere now by the means of thermal temperature checks. People with fever are subjected to further evaluation.

**Biomedical Waste Management of Covid-19**

The following measures are proposed for covid 19 waste management:

1. Dedicated sanitation workers deputed only to collect covid-19 waste
2. All the bags and containers from covid 19 areas should be labelled as “COVID-19 WASTE’
3. They should have foot operated lids.
4. The inner and outer surface of these containers must be disinfected daily with 1% sodium hypochlorite solution.
5. Double layers should be used to ensure no leaks.
6. Records of covid 19 waste should be maintained
Face shields, goggles, hazmat suit, nitrile waste and laboratory wastes such as transport media, plastic vials, vacuators, pipette tips should be disposed of in the RED bin.

Used masks such as N95 masks, head cover, shoe cover, disposable linen gown, leftover food, disposable plates, glasses, tissues, toiletries etc used by the patients should be disposed of in the YELLOW bin.

The risk of transmission of COVID 19 from sewage water of healthcare facilities is low but still requires adequate disinfection and waste water treatment.

Social distancing

Social distance turned out to be the most effective means in combating COVID 19 worldwide because it broke the chain of transmission by acting as a primordial level of prevention.

Primordial prevention is aimed at establishing and maintaining conditions that minimise hazards to health. This level of prevention acts even before the onset of the disease and this is particularly helpful for this pandemic because of the high number of asymptomatic people who are potential carriers and can spread the virus among others (8).

Primordial prevention is acting on the underlying socio-economic and environmental conditions leading to causation of disease. The most effective mode of intervention here is health education which is difficult in a country as diverse as India.

This is achieved by newspapers, local news channels, radio and the caller tune while dialing someone. These act as a constant reminder of maintaining the new normal as it is being called.

Here, the mode of transmission is droplet.

When the respiratory droplet particles are >5-10 micron in diameter they are referred to as respiratory droplets and when they are less than 5 micron they are called droplet nuclei.

According to the current information on Coronavirus, it is primarily transmitted between people through respiratory droplets and contact routes. The microorganisms that are less than 5 micron remain suspended in the air (9).

Factors that influence the airborne transmission:

1. Temperature
2. Humidity
3. Rainfall
4. Amount of sunlight
5. Wind
6. Human behaviour

Other factors include the socioeconomic and living conditions. The number of people residing in an area is important.

Indoor environments such as indoor ventilation, use of air conditioning also affects the spread of pathogens. Poor or inadequate ventilation has played an important role in the spread of many pathogens (10).

Since most of these factors are beyond our control, the only way to curtail the spread of such a pandemic that could be adapted was modifying the human behaviour that included social distancing, nation-wide lockdown, ban on large gatherings, schools and colleges, factories, malls, theatres and places of worship being shut and people were encouraged to stay indoors and practice work from home for as long as possible and to only go out for essential work.

This was important because it broke the chain of spread, decreased the number of people being exposed to the virus and also helped with contact tracing and thus reducing the burden on the health care professionals (11).

Social distancing norms suggest keeping a distance of about 6 feet or about 2 arms’ length from others. This is one of the easiest and most effective methods we have to avoid being exposed to the virus and slowing it’s spread locally and across the country and the world (3).

Just like hand hygiene, social distancing is an effective method based entirely on behavioural patterns of individuals and by not abiding to the norms of social distancing it could very easily overwhelm the healthcare system since the number of beds, the availability of ventilators, PPE kits, rapid tests for COVID19 and masks is limited.

Social distancing measures practiced in India currently:
1. Contact less delivery practices by food, grocery delivery services such as Swiggy

2. The number of people in a shop at a time is monitored.

3. Digital transactions are being preferred over cash.

4. Classes and meetings are being conducted over video calls.

5. Admissions are being done online.

6. Long queues outside of shops are avoided by keeping a safe distance between people. Shop owners have marked circles outside their shops as guides to maintain social distancing.

7. The number of people in a car is also according to the social distancing norms according to the various zones.

8. Restaurants have to maintain a distance of one meter between the tables and do regular temperature checks and follow proper hand hygiene.

9. Encouraging work from home as long as possible. (12)

10. Postponing all non-essential social and cultural gatherings. (3)

Nationwide lockdown

So the purpose of a nationwide lockdown was twofold: it slowed the transmission of the virus and second it also slowed the geographical spread of the virus which is extremely important in India. Both of these play a crucial role because slowing the transmission slows the short term spread of cases and by slowing the geographical spread and localizing the geographical spread there is flattening of curve in the long term. It is estimated that without the lockdown the cases would have been six times the current estimated infection.

Conclusion

COVID emerging as a pandemic has shed some light on a lot of aspects of the basics of health care such as hand washing. Adopting correct hand washing methods, teaching staff and students, knowing when to use soap and water and when a hand sanitizer should be used and focussing on the areas that are commonly missed while washing hands is important to know. Just knowing the right method won’t be enough, hospitals should also have the facilities and infrastructure to provide the same. These practices are cost efficient.

Some other lessons learnt from the pandemic

Social distancing and hand washing should become a part of normal life from now on whether there’s a virus or not and guidelines for people at risk people should be in place and practice.

Mental health is important.

We should pay more attention to our health.

Children and elderly who are more at risk to infections in general should not be neglected.

Hospitals should have infectious disease control teams with proper protocols in place.

Need to promote original R&D and strengthen our drug and vaccine development programme.

Conflict of Interest: Nil

Funding: DMIMS (DU), Wardha

Ethical Approval: From Institutional Ethical Committee, DMIMS.

References


5. Centers for Disease Control and Prevention


A Critical Appraisal of Inclusions of “Bioethics in Laboratory Medicine” into the Existing Postgraduate Curriculum of Pathology, Microbiology and Biochemistry

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Abstract

Introduction: Laboratory physicians confront with ethical issues in routine practice, but still ethics does not draw the importance it deserves. A critical appraisal of the existing postgraduate curriculum of Pathology, Microbiology and Biochemistry with reference to its Bioethical inclusions to their conformity with reference to Bioethical principles applicable to profession as included in UNESCO Declaration is needed.

Aim: To critically analyse the inclusions of “Bioethics in Laboratory Medicine” into the Existing Postgraduate Curriculum of Pathology, Microbiology and Biochemistry with reference to Bioethical principles incorporated in UNESCO Declaration

Material and Methods: It was a descriptive study for rapid review of literature for the purposes of identification and critical appraisal of bioethical considerations in Laboratory Medicine in existing Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology, Biochemistry with reference to Bioethical principles included in UNESCO declaration specially those applicable to profession and not propagation or advocacy.

Observations and Results: Upon critical analysis and mapping of the competencies in the Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology, Biochemistry with the principles in the UNESCO universal declaration, 16 competencies were identified as professional ethical inclusions. 19 Competencies suggested in regards to “Bioethics in Laboratory Medicine” for Guidelines for Competency Based Postgraduate Training Programme for MD In these subjects to make them commensurate with UNESCO universal declaration

Conclusion: This has brought out subsequent omissions which need to be managed in order to make competencies in Existing PG curriculum of Pathology, Microbiology and Biochemistry comparable with the UNESCO universal declaration and therefore, make it better and standardised on global and international level.

Keywords: Bioethics, Laboratory Medicine, Postgraduate Curriculum

Introduction

Medical practitioners are responsible towards their patients to exert their professional competence in a virtuous manner and to abide to the laws of the state & community. The general principle of healthcare ethics is that the patient’s welfare is paramount. This applies
to laboratory services as well. Similar to other fields of medicine, Laboratory Medicine is required to obey and practice high ethical specifications.

The qualified staff of a medical laboratory is restricted by the ethical principles of their corresponding profession. “Ethical medical practice is the expected conduct of laboratory physicians” and that striving to achieve high ethical standards is an essential aspect of medical excellence. Overarching goal for laboratory physicians is to maintain professional integrity.

Around 70% of medical diagnoses now rely on pathology laboratory analyses emphasizes the crucial role that laboratory physicians play in patient care. The clinician must be committed to same ethical fabric as the laboratory physician to assure that results of the investigations are enforced in the patient’s preferable interest. The clinician’s judgment about diagnosis, prognosis and treatment are routinely based on results and interpretations of laboratory investigations. Permanent damage to the patient may be caused by inaccurate tests and their faulty interpretations.

The term Bioethics was coined in 1926 by Fritz Jahr in article about a ‘bioethical imperative’ regarding the use of animals and plants in scientific research.

The United Nations Educational, Scientific and Cultural Organisation was established at Paris, France on 4th November, 1946. Hence, it was on the 19th of October, 2005 at the 33rd General Conference of UNESCO, which was held at Paris, that the Universal Declaration on Bioethics and Human Rights was adopted by one and all.

Taking stock of the overall global situation, the academic committee of the Medical Council of India formulated a detailed draft pertaining to competency based undergraduate medical education invoked a module titled ‘AETCOM Module’ (Attitude, Communication, and Ethics), which was notified in the year 2018 and is incorporated in the ‘Competency Based Undergraduate Medical Education Curriculum’. Such systematic guidelines regarding ethical considerations are missing in the existing postgraduate Medical curriculum of Pathology, Microbiology and Biochemistry.

Laboratory physicians confront with ethical issues in routine practice, but still ethics does not draw the importance it deserves. A report by the IFCC Task Force on Ethics indicates that formal and proper teaching of ethics is missing from many clinical chemistry and laboratory medicine training programs and that there is a perceived need for training programs for ethical considerations in laboratory Medicine.

It is in this context that a critical appraisal of Postgraduate curriculum of Pathology, Microbiology and Biochemistry with reference to its Bioethical inclusions vis-à-vis professional ethics inclusions needs to be looked into for the purposes of an judicious operational mix of the two, so that the learner is oriented on the said arena in an all-round manner so that optimal results thereto stand generated.

**Rationale Of The Study:**

In this context, the inclusions pertaining to ethical considerations in laboratory Medicine in postgraduate Medical curriculum of Pathology, Microbiology and Biochemistry in regard to their objective, scope, operation, outcome and relevance needs to be critically looked. A critical appraisal of the existing postgraduate curriculum of Pathology, Microbiology and Biochemistry with reference to its Bioethical inclusions to their conformity with reference to Bioethical principles applicable to profession as included in UNESCO Declaration is needed.

The appraisal of the Bioethics in Laboratory Medicine inclusion therein also become inevitably necessary to assess as to whether the said inclusions incorporate the tenets and principle of bioethics as have evolved over a period of time with reference to their applicability to professional ethics as applicable to modern medicine to be upheld in practicing of the same by the registered medical practitioners of the modern medicine.

**Research Question**

Are there any limitations and resultant scope for update for ethical considerations in the Laboratory Medicine in the existing postgraduate curriculum of Pathology, Microbiology and Biochemistry?
Aim

To critically analyse the inclusions of “Bioethics in Laboratory Medicine” into the Existing Postgraduate Curriculum of Pathology, Microbiology and Biochemistry with reference to Bioethical principles incorporated in UNESCO Declaration

Objectives:

1. To identify the bioethical inclusions in existing Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry

2. To critically analyze the bioethical inclusions in existing Guidelines in regard to their conformity with reference to Bioethical principles applicable to profession as included in UNESCO Declaration.

3. To suggest update and inclusions of “Bioethics in Laboratory Medicine” in these Guidelines so as to make them comparable with the Bioethical principles incorporated in UNESCO Declaration.

Material and Methods

Period of Study : Six Months

Design : Descriptive Study

Duration : October 19 to March 20

Procedure :

Rapid review of literature for the purposes of identification of bioethical considerations in Laboratory Medicine in existing Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry with reference to Bioethical principles included in UNESCO Declaration specially those applicable to profession and not propagation or advocacy.

Further, to critically appraise the conformity of the bioethical inclusions in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry with reference to their conformity with the bioethical principles as applicable to the profession in the UNESCO declaration.

Data:

Identifying Bioethical inclusions in existing Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry, which is already applicable to various medical colleges under the ambit of Medical Council of India.

Critical appraisal of the said identified Bioethical inclusions with reference to their conformity with the bioethical principles as applicable to the profession included in UNESCO declaration.

Further, to work out the inclusion of non-included bioethical principles in the form of structured competencies as “Bioethics in Laboratory Medicine” and their incorporation in these Guidelines with reference to their learning levels including their mode of assessment.

Collection Tool

Critical appraisal of the guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry, document against the Bioethical principles as applicable to profession in the UNESCO Universal Declaration.

Observations And Results

Amongst the UNESCO Universal Declaration, principles 3 to 20 were listed as professional bioethical inclusions which were directed towards the learner and his profession directly. The remaining principles were directed towards the states, international affairs and regulation i.e. advocacy. These were therefore excluded.

This study analyzed a total number of 16 competencies, enlisted in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry with their respective learning domains, levels as per Miller’s pyramid, and assessed in as against the principles
included in the UNESCO Universal Declaration laid down by the UNESCO.

These 16 competencies enlisted in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry reflected either single or multiple principles as per the UNESCO universal declaration of human rights and bioethics. Therefore these 16 competencies in Competency Based Postgraduate Training Programme were identified as professional ethical inclusions.

So, we had two sets – one set of competencies enlisted in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry and another set of UNESCO universal declaration principles.

Of these 16 competencies enlisted in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry, 4 competencies dealt with the cognitive domain and 12 competencies dealt with higher domains of learning – psychomotor and affective.

Article 3 – Human dignity and human rights was reflected in maximum number of competencies (n=12 competencies). This was followed by Article 4 – Benefit and harm (n= 8 competencies) and article 5 – autonomy and individual responsibility (n=8 competencies). Further, Article 14 – Social responsibility and health was noted to be reflected in 7 competencies (n=7 competencies) followed by article 10 – equality, justice and equity, article 11 – non-discrimination and non-stigmatization, article 12 – Respect for cultural diversity and pluralism, article 16 - Protecting future generations, article 18 – decision making and addressing bioethical issues were noted to be reflected in 3 competencies each (n=3 competencies). Article 8 – respect for human vulnerability and personal integrity, Article 13 – solidarity and cooperation, article 15 – Sharing of benefits issues were noted to be reflected in 2 competencies each (n=2 competencies). Article 6 – consent, article 7 – persons without the capacity to consent, and article 9 – privacy and confidentiality, article 20 – Risk assessment and management were noted to be reflected in 1 competency each. (n=1 competency).

However, article 17 – Protection of the environment, the biosphere and biodiversity article 19 – ethics committee had no reflection in the Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry.

**Discussion**

Upon critical analysis and mapping of the competencies in the Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology, Biochemistry with the principles in the UNESCO universal declaration, 16 competencies were identified as professional ethical inclusions. There are 2 principles in the UNESCO universal declaration which have no representation in the Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology, Biochemistry – articles 17 and 19. They are as under:-

Article 17 – Protection of the environment, the biosphere and biodiversity

Article 19 – Ethics committee

These principles should be adequately represented in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology, Biochemistry in order to make it commensurate it in conformity with the UNESCO universal declaration. These principles need to be articulated by working out their placement, learning level and modes of teaching and assessment.

The competencies framed for article 17 are two – first covering the cognitive domain with K and KH levels in the Miller’s pyramid and second covering the psychomotor and affective domains with S and SH levels in the Miller’s pyramid respectively. The competencies framed for article 19 are two – first covering the cognitive domain and second covering the psychomotor and affective domain with K, KH and S, SH levels in Miller’s pyramid respectively.

Addition of the suggested competencies would take into account the omissions realised in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry when critically appraised as against the UNESCO universal declaration of human rights and bioethics so
as to make it commensurate with the latter in the best way possible.

Protocols and guidance materials on ethical issues related to laboratory medicine have developed by many countries and professional agencies. For instance, the International Organization for Standardization (ISO) has created ISO 15189:2012 “Medical laboratories – Requirements for quality and competence”. Numerous professional organizations have outlined codes of ethics for clinical laboratory professionals. Despite importance of ethics in laboratory Medicine, there is variability in education that is focused on ethics in the laboratory Medicine.

So, there is a perceived need for systematic training programs for ethical considerations in laboratory Medicine in the existing Postgraduate Curriculum of Pathology, Microbiology and Biochemistry. Inclusions of “Bioethics in Laboratory Medicine” in systematic manner into the Existing Postgraduate Curriculum of Pathology, Microbiology and Biochemistry with reference to Bioethical principles incorporated in UNESCO Declaration will raise awareness with regards to various ethical dilemmas during their day to day working in Medical Laboratory.

The competencies framed under “Bioethics in Laboratory Medicine” covering the cognitive domain with K and KH levels in the Miller’s pyramid and remaining competencies covering the psychomotor and affective domains with S and SH levels in the Miller’s pyramid respectively.

Modes of teaching these competencies are didactic lectures as well as large and small group discussions, focussed group discussions. Modes of assessment for the cognitive domains can be in the form of short answer questions, long answer questions as a part of the respective theory examination. Modes of assessment of the psychomotor and affective domains can be in the form of viva voce, oral examination and clinical case presentation.

Conclusion

The present study deals with the mapping and matching of the competencies included in Guidelines for Competency Based Postgraduate Training Programme for MD in Pathology, Microbiology and Biochemistry with the UNESCO universal declaration on human rights and bioethics. This has brought out subsequent omissions which need to be managed in order to make competencies in Existing PG curriculum of Pathology, Microbiology and Biochemistry comparable with the UNESCO universal declaration and therefore, make it better and standardised on global and international level. These recommendations as suggested competencies pertaining to exclusions in Existing PG curriculum and addition of “Bioethics in Laboratory Medicine” can fulfil the realised omissions in the existing PG curriculum of these subject’s competencies.

Limitations

A detailed study of actualized operation and outcome thereto are beyond the scope and ambit of the present study taking into account the actualization of the suggested competencies thereto.

Ethical Clearance:

Institutional Ethical Committee Clearance was obtained from Institutional Ethical Committee of university.

Bibliography


Personal Hygiene Practices and Morbidity Pattern among A Tribal Primary School Children Of Maharashtra

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1Associate Professor, Department of Community Medicine, Shri Shankaracharya Institute of Medical Sciences, Bhilai, 2Associate Professor, Department of Forensic Medicine & Toxicology, Datta Meghe Medical College, Nagpur

Abstract

Background: Scholastic performance of the child depends upon the overall health of the child. Methods: A cross sectional study was conducted among 162 tribal school children. Results: Prevalence of anaemia was 54.9%. Second most common morbidity was dental caries (31.48%) followed by underweight (27.77%) Head louse infestation was common among girl students. Conclusions: Most common morbidities present among primary school children of a tribal school were anaemia, dental caries, underweight and refractive errors. Girl students had better hygiene than boys.

Keywords: Personal hygiene, morbidity, tribal, primary school

Introduction

The first school health check up service was started in 1909 in Baroda city of Gujarat.1 School going children should be healthy physical as well as mentally. If the health is sound then only they can devote their full time to studies. Unhygienic conditions make them vulnerable to various diseases. Morbidities among the school going children lead to absenteeism from the school. Sometimes it leads to school dropout also.

Provision of better health and free elementary education are enshrined in the constitution of India as the rights of the children .Health Education has important role in the improvement of the hygiene among the school children. Health education should be part of the routine teaching hours in the school. Students have less morbidities, if they are better in their hygiene status. Various strategies are launched to improve the hygiene among the school children. School sanitation and hygiene education (SSHE) is included in Surva Siksha Abhiyan (SSA).2 School should promote water sanitation and hygiene (WASH) strategy to improve the hygiene among the students.3

Objectives:

1. To assess the personal hygiene practices of tribal school children
2. To study the morbidity pattern among school children in a tribal school

Methodology

Study setting:
The study was conducted in a tribal school in one district of Maharashtra.

Study Period: Study was conducted from November 2019 to January 2020

Study design: Cross sectional study (descriptive study)

Inclusion criteria: students studying in 1st to 4th standard of a tribal school
Sample Size: Total on roll students from 1st to 4th standard were 177. 162 students were present on the day of school health check up, so 162 school children were included in the study. 89 boys and 73 girls were included in the study.

Sampling method: Convenience sampling

The study was conducted as a part of school health check up programme in a tribal school of Maharashtra. Students from the 1st to 4th standard were included in the study. Detailed examination of the school children was done by the faculties and residents of the Community Medicine department. Questions were asked regarding their personal hygiene practices. After the interview health education session was conducted regarding personal hygiene.

Preformed, pretested, pre-structured questionnaires were asked regarding their personal hygiene. Questionnaires were designed in local language. Face to face interview was conducted. Questions were asked regarding their hairs and nails cleaning, brushing of teeth, use of clean uniform, washing of hand after defecation, daily bathing and use of footwear.

The study was approved by the institutional ethics committee. Permission was taken from the head of the school for conducting health check up. All the data was kept confidential.

Statistical analysis: Statistical analysis was done by using R software version 3.6.1.

![Figure 1: Standard and sex wise distribution of students](image)

Results: Hygiene among the girl student was better than boys. [Table 1] The most common morbid conditions among primary school children attending tribal school were anaemia (54.93%) followed by dental caries (31.48%), underweight (27.77%) and refractive errors (14.81).[Table 2]
### Table No. 1: Personal hygiene practices among primary school children (n=162)

<table>
<thead>
<tr>
<th>Practices</th>
<th>Boys (%)</th>
<th>Girls (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair clean and combed properly</td>
<td>70 (78.65)</td>
<td>58 (79.45)</td>
<td>128 (79.01)</td>
</tr>
<tr>
<td>Nails clean and trimmed</td>
<td>72 (80.90)</td>
<td>65 (89.04)</td>
<td>137 (84.57)</td>
</tr>
<tr>
<td>Clean hands and feet</td>
<td>74 (83.15)</td>
<td>68 (93.15)</td>
<td>142 (87.65)</td>
</tr>
<tr>
<td>Clean teeth and oral cavity</td>
<td>69 (77.53)</td>
<td>63 (86.30)</td>
<td>132 (81.48)</td>
</tr>
<tr>
<td>Clean uniform</td>
<td>63 (70.79)</td>
<td>68 (93.15)</td>
<td>131 (80.86)</td>
</tr>
<tr>
<td>Use soap for washing hands after defecation</td>
<td>85 (95.51)</td>
<td>71 (97.26)</td>
<td>156 (96.30)</td>
</tr>
<tr>
<td>Use toothbrush/toothpaste/tooth powder for brushing of teeth</td>
<td>82 (92.13)</td>
<td>69 (94.52)</td>
<td>151 (93.21)</td>
</tr>
<tr>
<td>Brush teeth daily</td>
<td>89 (100)</td>
<td>73 (100)</td>
<td>162 (100)</td>
</tr>
<tr>
<td>Takes bath daily</td>
<td>87 (97.75)</td>
<td>73 (100)</td>
<td>160 (98.77)</td>
</tr>
<tr>
<td>Barefoot walking</td>
<td>9 (10.11)</td>
<td>3 (4.10)</td>
<td>12 (7.40)</td>
</tr>
<tr>
<td>Washing of hairs with shampoo (head bath)</td>
<td>71 (79.78)</td>
<td>59 (80.82)</td>
<td>130 (80.25)</td>
</tr>
</tbody>
</table>

### Table No. 2: Morbidities among school children (n=162)

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>Boys 89 (%)</th>
<th>Girls 73 (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>46 (51.68)</td>
<td>43 (58.9)</td>
<td>89 (54.93)</td>
</tr>
<tr>
<td>Injuries</td>
<td>8 (8.98)</td>
<td>2 (2.74)</td>
<td>10 (6.17)</td>
</tr>
<tr>
<td>Worm infestation</td>
<td>7 (7.86)</td>
<td>3 (4.10)</td>
<td>10 (6.17)</td>
</tr>
<tr>
<td>Fever</td>
<td>3 (3.37)</td>
<td>1 (1.37)</td>
<td>4 (2.47)</td>
</tr>
<tr>
<td>Refractive errors</td>
<td>14 (15.73)</td>
<td>10 (13.69)</td>
<td>24 (14.81)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>1 (1.12)</td>
<td>0 (0)</td>
<td>1 (0.62)</td>
</tr>
<tr>
<td>Underweight</td>
<td>24 (26.97)</td>
<td>21 (28.77)</td>
<td>45 (27.77)</td>
</tr>
<tr>
<td>Ear discharge</td>
<td>2 (2.25)</td>
<td>1 (1.37)</td>
<td>3 (1.85)</td>
</tr>
<tr>
<td>Skin problems</td>
<td>11 (12.36)</td>
<td>9 (12.33)</td>
<td>20 (12.35)</td>
</tr>
<tr>
<td>Dental caries</td>
<td>29 (32.58)</td>
<td>22 (30.14)</td>
<td>51 (31.48)</td>
</tr>
<tr>
<td>Louse infestation</td>
<td>2 (2.25)</td>
<td>15 (20.55)</td>
<td>17 (10.49)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>2 (2.25)</td>
<td>0 (0)</td>
<td>2 (1.23)</td>
</tr>
<tr>
<td>Cough and Cold</td>
<td>12 (13.48)</td>
<td>7 (9.59)</td>
<td>19 (11.73)</td>
</tr>
</tbody>
</table>
Discussion

This study was conducted as a part of school health check up. 162 students consisting of 89 boys and 73 girls were included in the study. Students from 1st to 4th standard were included in the study. [Figure 1] On the day of health check up 15 students were absent. Morbid conditions affect the attendance of the students.

Assessment of the hygiene practices was done using questionnaire. 79 % of the student’s hair were clean and combed properly. 84% student’s nails were clean and trimmed. 80% students were with clean uniform. 20% of the students do not wash their hairs regularly with shampoo. Head lice infestation is common among the girl students. All the students brush their teeth regularly. Seven percentages of the students do not wear footwear regularly. Prevalence of worm infestation was 6.17%. Worm infestation may be one of the causes for high prevalence of anaemia. 81% student’s teeth were clean. Seven percentage students do not use brush and toothpaste/tooth powder to brush their teeth. Boys have better hygiene status than girls found in a study conducted in Lahore also 94% have satisfactory hygienic status.6 64% school children do not use soap to wash their hand in Angolela, Ethopia.5 Health education has positive impact on hygiene status of the students. Before imparting health education, hygiene status of the students was poor among the students in a school in Lucknow.6 Hence, health education has a very vital role in the schools.

Prevalence of anaemia among the student was 54.93%. Anaemia was the most common morbid condition present among the students. Second most common morbidity was dental caries. Some students do not brush their teeth regularly. Teeth were dirty in 19% students. Fluorine deficiency causes dental caries. 27.77% of the students were underweight according to their age. Refractive error was present among 14.81% students. Refractive error affects visibility and consequently leaning capabilities of the students. Hence correction of refractive error should be done on priority basis so that it will not affect scholastic performance of the student. Some teachers may be trained to check refractive errors of the students.

Louse infestation was present in 20.55% girl students. Some girl students do not wash their hairs regularly with shampoo. 20% girl’s hairs were neither clean nor properly combed.12% students were having skin problems. Prevalence of worm infestation was 6.17%. Prevalence of cough and cold was 11.73%. Girl students were better in hygiene practices than boys.

Most common morbidities are anaemia, worm infestation and dental caries found in a study conducted in urban slum area of Hyderabad among school children aged 4 to 15 years. They also noticed poor hygiene among school children.7 Girl’s student knowledge regarding personal hygiene is better than boys in a school situated in slum area of Kolkata. In the same study, prevalence of lice and worm infestation is 40% and 45% respectively.8 Girls are better performer in hygiene. Most common morbid conditions present in the students are dental caries (65%), upper respiratory tract infection.9 Dental caries (38.9%) and worm infestation are commonly present among the rural students of Odisha. Only 10% students have poor hygiene status.10 Skin and dental diseases are common among tribal children above five years of age in Mysore district of Karnataka.11 Malnutrition, dental caries, worm infestation, skin diseases are common among school children.12 84% boys and girl students are malnourished in a tribal school of Thane district of Maharashtra. In the same study other most common morbidities are anaemia and dental caries.13 26.5% school children are malnourished in a school of a south India. Dental caries is present in 47% of the students found in the same study.14 Personal hygiene was better among girl students and most common morbidities were anaemia and worm infestation.15 Dental caries is common among urban school children than tribal and rural. Prevalence of dental caries among tribal school children is 15%.16

Unhygienic practices are common among tribal school children. It is one of the reasons for high prevalence of morbidities among them. It may lead to school absenteeism and affect academic performance.

Conclusions

Anaemia, dental caries and malnutrition were the most common morbid conditions present among primary school children of a tribal school. Hygiene status of the girl students was better than boys.
Recommendations:

Regular school health check up camp should be conducted to detect morbid conditions so as to reduce school absenteeism and improve their scholastic performance.

Conflict of Interest: None

Funding: Nil

Ethical Clearance: obtained from institutional ethics committee.

References

Diabetes in COVID-19: Management

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Abstract

The COVID-19 (Corona Virus Disease-19) has become the most critical health problem worldwide. It was first started from Wuhan, China and now it has affected more than 230 countries and millions of people. The diabetic patients with uncontrolled glycemic state for long term can result in many microvascular and macrovascular complications. Hence they are most commonly affected individuals during COVID-19. In this review we have briefed about the impact of COVID-19 in diabetes, the different pathophysiology and management of diabetes in this phase.

Key Words: COVID-19, Diabetes

Background

The Corona Virus Disease-19 (COVID-19) has changed the scenario all over the world. It ranges from affecting the children to geriatrics with several effects on economy, psychology, education and obviously the health of people.

It has started since December 2019 from Wuhan, China and progressed further involving more than 200 countries including India. [1][2][3] The COVID-19 is a fatal pandemic spread throughout the world. The corona virus is positive single stranded RNA, which is named so because of its similarity to the shape and structure of a crown or solar corona as per appearance in electron microscope. [1][3]

The WHO (World Health Organization) proclaimed the COVID-19 flare-up as international emergency crisis on 30th January, 2020 and moved up to Pandemic on eleventh March, 2020.[3][4]

It has caused many deaths and severe illnesses in many patients who have poor immunity. The vulnerable population regarding morbidity and mortality includes patients with presence of comorbid conditions. Diabetes is well known to affect the majority of population worldwide leading to comorbid condition. [1][5][6][7]

So we have written a brief review on the effect of COVID-19 on Management of Type 2 Diabetes patients.

Ethical Approval

This study did not warrant institutional review board review as no human subjects were involved.

Symptoms and Characteristics Of COVID-19

The symptoms of Corona Virus Infectious Disease-19 include fever, sneezing, coughing, body ache and difficulty in breathing. The incubation period varies from 2 to 14 days and the symptomatic phase from 6 to 41 days. Some of the patients may develop respiratory distress due to viral pneumonia and ultimately respiratory
failure. The number of hospitalizations is much more than common virus infection leading to severe respiratory distress and deaths. However, not all the patients progress towards respiratory distress and death, many people remain asymptomatic or with mild symptoms in spite of being corona positive. This is more dangerous as we cannot know where to identify and how to prevent the further spread. 

The Diabetes and COVID-19

Various studies conducted in china have shown that majority of the patients who were admitted due to COVID-19 were having comorbid conditions compared to patients with no comorbidity. The rate of death was higher in patients with comorbid state due to development of pneumonia. The diabetic individuals are with poor immunity due to increased blood sugar level, which acts as a factor contributing to invite COVID-19 infection easily. Among diabetic patients, exaggerated cytokine response leads to pro inflammatory response resulting in expanded degree of Interlekin-6 (IL-6) and C-responsive protein (CRP). The corona virus infection provokes inflammation and prompts a cytokine storm. This inflammatory process weakens the immunity of diabetic people putting them in vulnerable population group for COVID-19 infection.

Angiotensin Converting Enzyme 2 (ACE 2) receptors are present in pancreas, cell membrane of lung, enterocytes and many other tissues. ACE2 is involved in prevention of inflammation and has anti oxidant property. This enzyme degrades the angiontension II and I to smaller peptides, and these peptides play role as anti inflammatory and anti oxidants.

ACE 2 receptors can increase the blood sugar level even in patients who are not diabetic but when infected with COVID-19. This hyperglycemia may be due to damage to beta cells in pancreas. It was observed that patients infected with SARS were hyperglycaemic for 3 years even after recovered which establishes the injury to pancreatic beta cells. The study reported in Wuhan shows that the diabetic patients admitted for COVID-19 were unable to control the hyperglycemia in spite of management according to guidelines American Association of Clinical Endocrinologists and American Diabetes Associations. These all points add on the vulnerability of diabetic patients to catch on the COVID-19 infections and worsening of the morbid state leading to mortality.

Management of Hyperglycaemia in Diabetes with Presence of COVID-19

The diabetic patients who have uncontrolled blood sugar level for long term can cause many complications. A study reported that diabetes was the most impacted condition due to COVID-19 followed by chronic obstructive pulmonary disease (COPD), hypertension, heart disease, asthma, cancer and depression. So the diabetes leads among all the diseases to be affected specifically in presence of corona virus.

The COVID-19 deregulates the glycemic control in diabetic patients worsening the comorbid state of patient. A study reported in Wuhan that more than 50% of the blood glucose measurements were abnormal in admitted diabetic patients for COVID-19. Hence, it suggests the meticulous management of diabetic patients as hyperglycemia plays important role in organ damage in diabetes.

The type 2 diabetic patients have been most commonly managed by Metformin, and if it cannot be controlled with Metformin alone then additionally Insulin administration is required. The diabetics are at higher risk of hospital admission after catching corona virus infection. Therefore they need to be managed by Insulin administration for hyperglycemia when not controlled with oral antidiabetics.

The Metformin is first line drug to be prescribed to type 2 Diabetic individuals. The Metformin can significantly decrease the risk of mortality in patients with chronic respiratory infection. A study reported there was significant decrease in death rate in diabetic patients with Metforin compared to diabetic without Metformin.

It activates the AMP-activated protein kinase (AMPK) in the liver, which causes functional changes to ACE 2 receptor. Thus, it may diminish the attachment of SARS-CoV-2. So Metformin seems to be helpful to diabetics during COVID-19.
The other antidiabetic drugs which are commonly used include GLP-1 analogue (Glucagon-Like-Peptide-1 analogue), SGLT-2 (Sodium Glucose Transporter-2) blockers and DPP4 (Dipeptidyl Peptidase 4) inhibitors. Besides the antidiabetic effect, they play role as anti-inflammatory, anti adipogenic and antagonism of Insulin Resistance. However, SGLT2 inhibitors and GLP1 analogue increase the expression of ACE2 receptors, so they can worsen the state of patient in presence of COVID-19. So it seems to withhold the SGLT 2 inhibitors in patients at this phase when COVID is widely spread. [14]

DPP4 inhibitors can increase the chances of upper respiratory infection but not the pneumonia. However, enough clinical evidences are needed to establish the role of DPP4 inhibitors in diabetic patients with COVID-19. The preclinical data has revealed that DPP4 inhibitors can reduce the severity of COVID-19 but again the sufficient clinical evidence is must to ascertain the relationship. [14][18]

A study reported that Thiazolidinediones put the diabetic patient at higher risk of pneumonia in comparison of patients on sulfonylureas. A study had reported that Pioglitazone increase the expression of ACE 2 receptors and so it may worsen the metabolic state in presence of COVID-19. [14][18]

**Conclusion**

The COVID-19 has become the serious health problem with world wide spread. The vulnerable population to be affected with it and leading to morbidity and mortality includes patients with poor immunity and comorbid conditions. The diabetic individuals are most commonly impacted with COVID-19 and so their strict control of hyperglycemia is mandatory to prevent the further worsening of state. Metformin has been the first choice of drugs to be prescribed in Type 2 diabetes. It has additional anti inflammatory property and it significantly reduces the morbidity as well as mortality in type 2 diabetic patients in comparison of other antidiabetic agents during this phase.

**Research Funding:** None

**Financial support and sponsorship:** Nil

**Conflict of Interest:** None

**Ethical Clearance:** It was obtained from Sumandeept Vidyapeeth Institutional Ethics Committee before starting the study.

**References**


The Effect of Communication Ability of Care Workers in Charge of Visiting Care in Elderly at-Home Welfare Centers on Quality of Service: with Empathy as Mediator

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Abstract

**Background/Objectives:** The purpose of this study is to provide data for nursing intervention development that increases the quality of service of care workers by identifying the mediating effect of empathy in the effect of communication ability on quality of service.

**Methods/Statistical analysis:** Data were collected for 93 visiting care workers. Data were analyzed by descriptive statistics, Pearson’s correlational coefficients, multiple regression and Sobel’s test.

**Findings:** Subjects’ quality of service and communication ability (r=.62, p<.001), and empathy (r=.56, p<.001) showed high positive correlation. As a result of the mediating effect of empathy, communication ability had a significant effect on empathy in step 1 (β=.64), in step 2, communication ability affected the quality of service (β=.62), in step 3, communication ability had a positive effect on the quality of service (β=.45), and empathy also had a positive effect on quality of service (β=.28) and showed 42.2% explanatory power. It was found that empathy had a partial mediating effect.

**Improvements/Applications:** Care workers should make efforts to provide services by understanding and listening to and empathizing with the expressions of the elderly. The directors of the visiting care center should develop and train programs that can improve effective communication, empathy and quality of service.

**Keywords:** Care workers, Mediating effect, Communication Ability, Empathy, Quality of Service

Introduction

In Korea, with the start of Long-Term Care Insurance for the Elderly in 2008, the health and welfare of the elderly are improving as the elderly care policy is expanded and the working field is revitalized in taking care of the elderly through the expansion of care personnel such as care workers as well as medical personnel. In addition, the rapid increase of the elderly population along with the prolongation of life expectancy, coupled with the active social advancement of women, is raising the need for more elderly care workers.

Care workers for the elderly care for the elderly in elderly nursing hospitals or belong to a visiting care center and are active in the form of caring for the elderly in elderly families in the form of visiting care. In particular, care workers who are in charge of visiting care directly and proactively provide elderly care, so their role is considered to play a very important and decisive role in the elderly’s daily activities and health.

On the other hand, looking at the proportion of the population by age in 2017 and 2067, the proportion of the aged 65 or older will increase (13.8% → 46.5%), and
as it will reach 7.07 million in 2017, 10 million in 2025, and 19.01 million in 2050 [1], care for the elderly will become more and more important. Among the elderly in Korea, 89.5% of the total elderly responded that they have been continuously suffering from the disease for more than 3 months and have chronic diseases, and elderly people in general have more than two chronic diseases on average, and 83.5% of all elderly people have taken prescription drugs for more than 3 months. In addition, the physical and cognitive functions of the elderly are also limited as they age, and 71.4% of the subjects with reduced physical function are being cared for. In addition, because more than half of them rate their health as bad [2], it is thought that more care for the elderly, that is, nursing services, will be needed.

Quality of elderly care service refers to the excellence of services provided to pursue user satisfaction [3]. Quality of service is measured by considering reliability, responsiveness, assurance, empathy, and tangible dimensions. Therefore, the quality of service of care workers can be an index for evaluating the visiting care of the elderly, and the excellent quality of service of care workers will have a great effect in maintaining health while increasing the satisfaction of the elderly.

In order to improve the quality of service of care workers, it is necessary to identify the factors that affect it. Previous studies have shown that humans have the ability to live socially through communication. Humans with such communication ability can communicate their opinions efficiently and convincingly and maintain a smooth relationship with others, which is an important factor in determining the quality of human life [4]. Jung [5] reported that communication ability is a factor affecting the quality of care. For communication ability, the better you empathize, the better you can demonstrate social competence in various situations [6] and it can be said that the quality of service of care workers can be improved. In addition, empathy, which can understand and feel the inner experiences of others, such as emotions and psychological states, is closely related to communication ability [7] and it is said that by predicting the other’s behavior through the ability to accept and understand the other’s role, it can respond appropriately to social situations, thereby promoting interaction and communication ability [8].

Therefore, it was to provide basic data for the development of programs that can improve the quality of service of care workers by testing the mediating effect of empathy in the relationship between communication ability and quality of service.

Methods

1. Subjects

The subjects were 93 care workers belonging to an elderly at-home welfare center in D City, and care workers receiving refresher education in education centers in S and G City. They were adult who understand the purpose of the study and voluntarily expressed their willingness to participate and gave written consent, with working period of more than 6 months. Using the G-power 3.1.9.4 program, 88 people were calculated as a result of calculating the number of samples required to maintain 2 predictors, .15 effect size, .05 significance level, and .90 power, and in consideration of the 10% dropout rate, 96 people were surveyed, and the data of 93 people were finally analyzed.

2. Instruments

2.1. Communication ability

The comprehensive measure of interpersonal communication ability developed by Hue [9] modified and supplemented by Bae [10] was used. With a total of 14 questions, 2 items were coded in reverse. The questions were on a Likert 5-point scale, and higher the score, the higher the communication ability. At the time of development, the reliability Cronbach’s α was .72, and in this study, it was .83.

2.2. Empathy

The tool for empathy was a self-report test developed by Davis used by Lee [11]. This tool consists of a total of 26 questions, consisting of 14 questions of cognitive empathy and 12 questions of emotional empathy. The questions were on a Likert 5-point scale, and 8 inverse questions were coded in reverse. The higher the score, the higher the empathy. The reliability Cronbach’s α in the study by Lee [11] was .72 and in this study was .85.

2.3. Quality of service

The ‘SERVQUAL’ scale developed by Parasuraman,
Zeithmal, Berry\textsuperscript{[12]} used for care workers by Jung\textsuperscript{[5]} was used. It has a total of 20 questions on the Likert 5-point scale, and higher the score, the higher the quality of service. In the study of Jung\textsuperscript{[5]}, the reliability of the tool Cronbach’s $\alpha$ was .97 and in this study was .92.

3. Data collection

Data collection was from August to October 2020. Researchers and research assistants visited one elderly at-home welfare center located in the east area of D City, each one care worker maintenance training institution in G and S City, and after obtaining permission to the center director and the director, with the help of a research assistant who was educated on the research objectives and methods, he directly explained the research objectives to the care workers and received written consent. Data was collected through completing the questionnaires. The time required to complete the questionnaire was 10~15 minutes.

4. Ethical consideration

This study was approved by K University’s Institutional Review Board (KNU_IRB_2020-62).

5. Data Analysis

Using the SPSS/WIN 23.0 program, the descriptive statistics of the general characteristics and variable were calculated, the correlation between each variable was calculated using Pearson’s correlation coefficients, and the mediating effect of empathy in the relationship between communication ability and service quality utilized multiple regression. The significance test for mediating effect size was analyzed by Sobel’s test.

Result and Discussion

1. General characteristics of subjects

In the general characteristics of the subjects, the subjects of this study were care workers (100%) who are in charge of visiting care, ranging in age from 39 to 82 years old, with an average of 58.34±7.59 years old and 58.1% (54 persons) under 59 years old. Most (92, 98.9%) were women, and as for the educational background, 68.6% (64 people) had a high school diploma or higher, and the average working experience as a care worker was 40.48±34.85 months, ranging from 6 months to 12 years. It was found that more than half (64.5%, 60 people) had no other certifications than the care worker certification. 39 (41.9%) care workers had received maintenance training or job training once in the past year. For monthly income as care workers, about 0.51 million to 1 million won accounted for 47.3% (44 people).

2. Degree of communication ability, empathy and quality of service in subjects

The care workers’ communication ability scored 3.80 points out of 5 points, empathy scored 3.68 points out of 5 points, and quality of service scored 4.20 points out of 5 points [Table 1].

![Table 1. Degree of communication ability, Empathy and Quality of service in Subjects (N=93)](image)

3. Correlation between communication ability, empathy, and quality of service in subjects

Quality of service and communication ability ($r=.62, p<.001$), quality of service and empathy ($r=.56, p<.001$), and empathy and communication ability ($r=.64, p<.001$) all showed high positive correlation [Table 2].
Table 2. Correlation between Communication Ability, Empathy, and Quality of Service in Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Communication ability r(p)</th>
<th>Empathy r(p)</th>
<th>Quality of service r(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication ability</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empathy</td>
<td>.64 (&lt;.001)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Quality of service</td>
<td>.62 (&lt;.001)</td>
<td>.56 (&lt;.001)</td>
<td>1</td>
</tr>
</tbody>
</table>

4. Mediating effects of empathy in the relation between communication ability and quality of service in subjects

As a result of examining the autocorrelation of the dependent variable and the multicollinearity between the independent variable before testing the mediating effect, the Durbin-Watson index for autocorrelation was 1.59-1.77, which was close to 2, which was independent. The multicollinearity between the independent variables was less than 10 with the VIF (Variation Inflation Factor) index 1.00~1.71, and tolerance was 0.59~1.0, which is above the standard value of 0.1, and there was no multicollinearity, which meant the data was suitable for regression analysis.

As a result of applying the mediating effect, in step 1 regression analysis, communication ability, an independent variable, had a statistically significant effect on empathy, a mediating variable (β=.64), and the explanatory power for empathy was 40.8%. In the second-stage regression analysis, communication ability, an independent variable, had a significant effect on the quality of service, a dependent variable (β=.62), and the explanatory power for quality of service was 38.3%. In step 3, in order to test the effect of empathy, a mediating variable, on quality of service, which is a dependent variable, as a result of regression analysis with communication ability and empathy as predictive factors and quality of service as dependent variable, communication ability was found to have a positive effect on quality of service (β=.45), and empathy also had a positive effect on quality of service (β=.28), and it showed 42.2% of explanatory power.

As a result of comparing the β values, it was confirmed that empathy showed a partial mediating effect as much as 0.17 since the β=.62 value in step 2 was larger than the β=.45 value in step 3. As a result of confirming the significance of the mediating effect coefficient, it was statistically significant (Z=2.53, p=.011) [Table 3].

Table 3. Mediating effects of empathy in the relation between communication ability and quality of service

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>β</th>
<th>t</th>
<th>p</th>
<th>Adj. R2</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step1: Communication ability → Empathy</td>
<td>.63</td>
<td>.64</td>
<td>8.03</td>
<td>&lt;.001</td>
<td>.408</td>
<td>64.45</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Step2: Communication ability → Quality of service</td>
<td>.61</td>
<td>.62</td>
<td>7.62</td>
<td>&lt;.001</td>
<td>.383</td>
<td>58.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Step3: Communication ability, Empathy → Quality of service</td>
<td>.422</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34.53</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1. Communication ability → Quality of service</td>
<td>.44</td>
<td>.45</td>
<td>4.30</td>
<td>&lt;.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Empathy → Quality of service</td>
<td>.28</td>
<td>.28</td>
<td>2.67</td>
<td>.009</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Z= 2.53, p=.011
Discussion

The purpose was to analyze the relationship between communication ability, empathy and quality of service of care workers in charge of visiting care, and to understand the mediating effect of empathy in the effect of their relationship and communication ability on quality of service.

The communication ability of this study was 3.80 out of 5 points, higher than 3.48 points of hospital nurses\(^{[10]}\) and higher than 3.29 points of nursing hospital care workers and home visit care workers\(^{[5]}\). This is because the care workers in this study are care workers who are in charge of home visits, and because they provide elderly care services by themselves, it is difficult to perform their duties well without proactive and active communication, and it is thought that communication ability scored higher than that of health care workers working in other fields or in other occupations. The level of empathy was 3.68 points, similar to the empathy of nursing students 3.39\(^{[13]}\) and the emotional intelligence of care workers 3.53, and it can be said that care workers understand the psychology and attitude of others, feel compassion, care for them, and feel the pain of others’ difficulties as well as have an attitude to empathize.

The service quality of care workers was 4.20 out of 5, which was higher than that of 3.80 of Jung\(^{[5]}\). It is expected to provide training to continue to provide quality service.

The relationship between the subjects’ communication ability, empathy and quality of service showed a high positive correlation. This was consistent with the results\(^{[5]}\) showing positive correlations with communication ability, emotional intelligence, and quality of care services of 258 care workers. The higher the communication ability and empathy, the better the quality of service. It is desirable to provide a training program for care workers to effectively learn verbal and non-verbal communication skills and have the emotional ability to empathize.

Empathy showed partial mediating effect in the relationship between communication ability and quality of service of care workers. These results were similar to the results that sympathy had a mediating effect in the communication ability and interpersonal relationship of nursing students\(^{[13]}\) and that the communication ability and emotional intelligence of care workers had an effect on service quality\(^{[5]}\). In hospital nurses, communication ability was found to be a factor affecting nursing work performance, and hotel bakery employees’ emotional intelligence and empathy showed a high correlation with service quality, and it was similar to the report that empathy had a moderating function in the relationship between emotional intelligence and job satisfaction\(^{[14]}\).

By applying the result that empathy and emotional abilities affect service quality in hotel employees, like nursing care workers as a service occupation, and applying it to the service quality of care workers, practice should be pursued in order to improve communication skills and empathy of them.

Conclusion

Based on the results of this study, it is necessary to increase self-leadership in order to increase the job competency of care workers in charge of visiting care. To do so, self-control and self-motivating autonomy need to be increased, the role and abilities should be realized through thinking and action strategies to set goals and to take responsibility for the results. Ultimately, such efforts can increase the influence of care workers, and it is thought that they can improve their job competency and increase the satisfaction of the care target. Therefore, it is suggested that a leadership improvement program be developed and actively used as a refresher education for care workers.

Ethical Clearance: Not required

Source of Funding: Self

Conflict of Interest: Nil

References

3. Lee JS. A Study on the Effect of Job Satisfaction of Care-Givers on the Quality of Service. Master


Assessing the Training Needs Regarding ‘Breaking Bad News’ amongst Emergency Medical Services (EMS) Professionals in Pune India

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Abstract

Introduction: ‘Breaking Bad News’ (BBN) in the scenario of poor prognosis or death is considered to be one of the most stressful aspects of the duties of a Healthcare professional. In the recent years, the subject of BBN has gained traction among healthcare professionals and psychologist leading to the creation of some comprehensive models to train professionals. Emergency Medical Professionals are among the likeliest to encounter BBN situations. Hence it is imperative that they be formally trained to deal with patients and their families effectively.

Objective: To assess the training needs regarding ‘Breaking Bad News’ (BBN); amongst Emergency Medical Services (EMS) Professionals.

Methodology: The study was conducted amongst 150 Emergency Medical Professionals in Pune, India. A previously tested and validated questionnaire developed by Rasmus et al in June 2020 was utilized for the study. Questionnaire was administered to the respondents through online mode after obtaining informed consent. Responding to all questions was mandatory. The respondents were given one day to fill the questionnaire. Any queries pertaining to questionnaire were clarified during data collection.

Discussion: The present study was done to assess the baseline awareness of EMS professionals about BBN and to devise a customized training program for them. Unfortunately, majority of the respondents were not even aware that BBN is a separate subject having specific procedures and protocols followed globally. The lack of training instils fear of facing such situations and adversely affects the mental health of EMS professionals. This can be corrected by utilizing tools like simulation and role play to provide adequate exposure to EMS professionals in a safe environment.

Conclusion: The findings of the study point towards an emergent need of formal practical training on this crucial yet ignored aspect of the health care professionals’ duties. Comparative Studies can be conducted in the future to assess the effectiveness of this training program.

Keywords: Breaking Bad News (BBN), Emergency Medical Services (EMS) professionals, Formal training, Training Needs

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Introduction

‘Breaking Bad News’ (BBN) in the scenario of poor prognosis or death is considered to be one of the most stressful aspects of the duties of a Healthcare
professional. Unfortunately, the healthcare education curriculum does not provide for formal training regarding effective communication with the patient and/or caregivers.¹

A study in 2010 conducted amongst physician and nurses in Iran by Arbabi et al,² concluded that lack of adequate communication skills among healthcare professionals leads to inertia in disclosing bad news to the patients or their relatives. This was partly attributed to the fear of managing the adverse emotional reaction of the patients’ or caregivers after listening to bad news.

In the recent years, the subject of BBN has gained traction among healthcare professionals and psychologist leading to the creation of some comprehensive models to train professionals. A few of these models that has gained popularity include the ABCDE model developed by Rabow and Mephee. This acronym stands for Advance preparation, Building a therapeutic relationship or environment, communicating well, Dealing with patient and family reactions, and Encouraging and validating emotions.³

Another model called SPIKES has been recognized in standard guidelines in numerous countries.⁴⁵ The model consists of six steps including: Setting up the interview, assessing patient’s Perception, obtaining Patient’s information, Providing, Knowledge and information to the patient; addressing the patient’s Emotions with empathic responses and Strategy and Summary⁴.

Although these models offer a structured approach for healthcare professionals to adopt, their utilization has been abysmally low. One way to improve their utilization is to sensitize healthcare professionals on the significance of effective communication during an adverse outcome. Physician and caregiver surveys have highlighted the importance of upfront and honest communication while delivering bad news⁶⁷⁸.

Within the healthcare cadre, Emergency Medical Professionals are among the likeliest to encounter BBN situations. Hence, it is imperative that they be formally trained to deal with patients and their families effectively.⁹ The first step in this direction is to assess the training needs to help tailor practical training program based on recognized protocols.

**Objective:** To assess the training needs regarding ‘Breaking Bad News’ (BBN); amongst Emergency Medical Services (EMS) Professionals.

**Methodology**

The study was conducted amongst 150 Emergency Medical Professionals in Pune, India. A previously tested and validated questionnaire developed by Rasmus et al in June 2020 was utilized for the study.

The original questionnaire comprised of two sections wherein the first section recorded sociodemographic information. The second section consisted of 7 items pertaining to the exposure of the respondents in BBN.

Of the 7 items 3 were required to be answered in a binary Yes/No and the remaining 4 items were in the format of Multiple Choice Questions (MCQ).

Questionnaire was administered to the respondents through online mode after obtaining informed consent. Responding to all questions was mandatory. The respondents were given one day to fill the questionnaire. Any queries pertaining to questionnaire were clarified during data collection.

All 150 respondents reverted with the completely filled questionnaire. The data was tabulated and statistically analysed with the help SPSS version 23.

**Results**

On the basis of the demographic findings, it was seen that the average respondents were between the age group of 21-25 years with 84% female majority. Further, 42% were BHMS graduates and more than 93% of the respondents had a work experience of 1-5 years.
As seen in figure 1 and 2, 3% of the respondents had never participated in a situation in which they had to deliver poor prognosis or news regarding death of a patient. Within the rest nearly 50% of the respondents had to face BBN situations regularly.
Figure 3.
As seen in figure 3, majority of the respondents were not aware of any specific procedure/protocol required to be followed during a BBN situation.

Figure 4.
As seen in figure 4, only one fifth of the respondents had undergone formal training in BBN as part of their academic curriculum. As many as, 62% of the respondents cited that that they have not received any kind of formal/informal training on the subject.
As seen in figure 5, majority respondents felt that using easy and understandable language is the most important rule while delivering bad news.

As shown in figure 6, a whopping 77% respondents felt that dealing with the patients’ emotions was the most challenging and difficult aspect.
**Discussion**

The present study was done to assess the baseline awareness of EMS professionals about BBN and to devise a customized training program for them. Most of the respondents were in the age group of 21-25 with nearly 93% of them having a work experience of 1-5 Years. This category of EMS professionals is the most vulnerable to poor communication and inept handling of difficult patient situations. Being first responders, it is essential to train them in various soft skills including empathy and effective communication apart from hard clinical skills.

Unfortunately, majority of the respondents were not even aware that BBN is a separate subject having specific procedures and protocols followed globally. This in spite of the fact that majority of them were encountering BBN situations regularly in course of their work. The higher frequency of such situations could be partly attributed to the ongoing Covid 19 pandemic.

Current teaching curricula do not adequately focus on soft skills teaching and such delicate topics are seldom covered in continued medical education courses and conferences. This trend needs to be changed urgently so that EMS professionals are better equipped in handling stressful situations like bad prognosis or death of the patient.

Even in the absence of formal training the respondents were aware of the basic individual rules to use while delivering bad news. Although, the lack of training reflected in the meagre 3% correct response to the item which is that effective communication requires eye contact, empathy, finding a right place to deliver bad news and using an understandable language to deliver bad news.

The lack of training instils fear of facing such situations and adversely affects the mental health of EMS professionals. This can be corrected by utilizing tools like simulation and role play to provide adequate exposure to EMS professionals in a safe environment.

**Conclusion**

The findings of the study point towards an emergent need of formal practical training on this crucial yet ignored aspect of the health care professionals’ duties. Comparative Studies can be conducted in the future to assess the effectiveness of this training program.

**Acknowledgement:** We thank Rasmus et al for granting permission to use the questionnaire for the research study.

**Conflict of Interest:** None

**Source of Funding:** Self

**Ethical Clearance:** Obtained from IEC, SIU

**References**

Assessment of Awareness and Preparedness regarding Management of Major Traumatic Haemorrhage in Prehospital Settings amongst EMS Professionals, Pune, India

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Abstract

Background: In trauma cases acute major hemorrhage in the first few hours of injury is the leading cause of death in as many as 30% – 40% cases. Major hemorrhage can result in the collapse of the circulatory system and the patient can present to the emergency room in a state of shock. EMS professionals are expected to have high degree of awareness as well as should possess the requisite skills and preparedness in the various techniques utilized to control life threatening hemorrhage.

Aim: To assess the Awareness and Preparedness regarding management of major traumatic hemorrhage in prehospital settings among EMS students.

Methodology: The study was conducted amongst 141 Emergency Medical Service professionals. The original questionnaire consisted of 2 parts, first containing two clinical scenarios testing the awareness level and the second one containing twenty four items pertaining to the preparedness of EMS professionals in major traumatic hemorrhagic situations.

Result: The mean score obtained for the parameter awareness was 18.32 out of maximum score of 28. Respondents scored well on the parameter pertaining to the management of venous bleeding and amputated limb while scored relatively lower on the items pertaining to the management of capillary bleeding.

Conclusion: The present study provides encouraging results with regards on the awareness and preparedness levels of EMS professionals. It also provides insight into future training needs to enhance preparedness in cases of infrequent hemorrhagic emergencies.

Keywords: Haemorrhage, Amputated Limb, Venous, Capillary.
and deft management of the Airway, Breathing and Circulation in the patient\textsuperscript{4}

Kauvar \textit{et al} \textsuperscript{5}(2005) in a study emphasized the significance of appropriate triage, early transportation to trauma center, efficient communication along with proper bleeding control steps for effective management of trauma cases.

The corner stone of management in such cases is the effective control of hemorrhage. This requires thorough understanding of the pathophysiology of hemorrhage and presence of mind on part of the Emergency Medical Services professionals. Success in hemorrhage control depends on effective communication between EMS professionals and consultants and strict adherence to guidelines of hemorrhage control.\textsuperscript{5}

The role of EMS professionals is critical as most cases of trauma injury die due to major hemorrhage even before they reach the hospital.\textsuperscript{6}

Hence EMS professionals are expected to have high degree of awareness of the pathophysiology and management of major traumatic hemorrhage as well as possess the requisite skills and preparedness in the various techniques utilized to control life threatening hemorrhage.

**Aim**

To assess the Awareness and Preparedness regarding management of major traumatic hemorrhage in prehospital settings amongst EMS professionals.

**Methodology**

The study was conducted amongst 141 Emergency Medical Service professionals at Symbiosis Center for Health Skills, Pune, India.

A pretested and validated questionnaire developed by Mofrad \textit{et al}\textsuperscript{7} was utilized for the study.

The original questionnaire consisted of two parts, first containing two clinical scenarios testing the awareness level and the second one containing twenty four items pertaining to the preparedness of EMS professionals in major traumatic hemorrhagic situations.

Within the twenty four items, four items dealt with the management of capillary bleeding, six with venous bleeding, ten with arterial bleeding and four with amputation of the limb.

Each clinical scenario consisted of Multiple Choice Questions with four correct options and four incorrect options. Each correct option marked by the respondent was given a score of one. The maximum score that could be obtained in the first section of awareness was twenty eight (Maximum score of four in each of the seven MCQs).

Preparedness was graded on a three point Likert scale ranging from Cannot do (Score 0), Can do but not perfectly (Score 1) and Can do perfectly (Score 2).The maximum score that could be obtained was eight for management of capillary bleeding, twelve for management of venous bleeding, twenty for management of arterial bleeding and eight for amputation of the limb.

Written informed consent was obtained from all the respondents before administration of the questionnaire. The questionnaire was emailed to the respondents and they were given a time of one day to revert back with the completely filled questionnaire.

The data collected was tabulated and statistically analyzed using SPSS version 23.

**Result**

<table>
<thead>
<tr>
<th>Percentage wise Distribution of Age</th>
<th>Age (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 to 25 years</td>
<td>60.3%</td>
</tr>
<tr>
<td>26 to 30 years</td>
<td>30.5%</td>
</tr>
<tr>
<td>31 to 35 years</td>
<td>5.7%</td>
</tr>
</tbody>
</table>
Cont... Table 1. Demographic data frequency distribution table

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 to 40 years</td>
<td>2.1%</td>
</tr>
<tr>
<td>Above 40 years</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage wise Distribution of Sex</th>
<th>Sex (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>67.4%</td>
</tr>
<tr>
<td>Female</td>
<td>32.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experience</th>
<th>Experience (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 year</td>
<td>39.7%</td>
</tr>
<tr>
<td>1 to 2 year</td>
<td>35.5%</td>
</tr>
<tr>
<td>2 to 3 year</td>
<td>14.2%</td>
</tr>
<tr>
<td>Above 3 year</td>
<td>10.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage wise Distribution of Qualification</th>
<th>Qualification (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBBS</td>
<td>0.7%</td>
</tr>
<tr>
<td>BAMS</td>
<td>34.8%</td>
</tr>
<tr>
<td>BHMS</td>
<td>44.7%</td>
</tr>
<tr>
<td>BUMS</td>
<td>16.3%</td>
</tr>
<tr>
<td>OTHERS</td>
<td>3.5%</td>
</tr>
</tbody>
</table>

Majority of the respondents belong to the age group of 21-25 years with experience of less than 2 years.

Table 2. Mean and S.D of the awareness score.

<table>
<thead>
<tr>
<th>Total Average Score Scenario 1</th>
<th>Total Average Score Scenario 2</th>
<th>Total Average Score Of Both The Scenarios</th>
<th>Standard Deviation –</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.56</td>
<td>7.76</td>
<td>18.32</td>
<td>2.04</td>
</tr>
</tbody>
</table>

The mean score obtained was 18.32 out of maximum score of 28.
Fig 1. Percentage mean score of Preparedness

As seen in Fig 1. The respondents scored well on the parameter pertaining to the management of venous bleeding and amputated limb while scored relatively lower on the items pertaining to the management of capillary bleeding.

**Discussion**

The present study was aimed at assessing the level of awareness and preparedness with regards to the management of major traumatic haemorrhage amongst working EMS professionals.

Although the EMS professionals in the study has limited years of work experience they possessed above average level of awareness regarding the clinical presentation of major traumatic haemorrhage. The respondents scored better on the questions related to the symptomatology and diagnosis but scored relatively lower on the questions testing awareness of management principles.

In terms of practical preparedness the respondents scored relatively better and showed confidence in the skills related to various interventions required in a case of major traumatic haemorrhage. Within the four kinds of haemorrhage scenario the responses reflected least preparedness in the emergency management of capillary bleeding. The respondents showed greater degree of confidence and preparedness in management of venous bleeding, arterial bleeding and amputated limb. The lower score obtained of capillary bleeding could be attributed to lack of adequate training or the relative low frequency of such emergencies in clinical practice. Nevertheless the data points towards the need of reinforcement and regular training in all kinds of traumatic haemorrhage to further boost the preparedness of EMS professionals.

Many causes of major traumatic haemorrhage are relatively rare yet life threatening as they occur seldom in clinical practice the EMS professionals failed to get adequate on-the-job-training for such emergencies. Use of clinical simulation is an effective way of training EMS professionals in rare hemorrhagic emergencies. With the utilization of high fidelity manikins and part task trainers scenarios can be created and training can be provided in safe environment.

**Conclusion**

Major traumatic hemorrhage is an extremely important and frequent emergency encountered by EMS professionals. The present study provides encouraging results with regards on the awareness and preparedness levels of EMS professionals. It also provides insight into future training needs to enhance preparedness in cases of infrequent hemorrhagic emergencies.

**Conflict of Interest:** None

**Source of Funding:** Self
Ethical Clearance: Obtained from IEC, SIU

References

To Assess Awareness Regarding Child Abuse and Neglect, amongst Emergency Medical Professionals in Pune, India – A Pilot Study

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Abstract

Introduction: Violence against children is widespread and affects millions of children globally. Violence against children occurs in various forms including physical violence, maltreatment, bullying, emotional or psychological violence, forced and bonded labour, and trafficking.

To prevent child abuse, it is essential that a high degree of awareness is essential amongst all stakeholders including parents, educators and medical professionals. The common thread among all studies conducted in medical professionals is the lack of focused training and sensitisation that can enhance the ability to detect such cases and respond to them promptly.

Objective: To Assess Awareness Regarding Child Abuse and Neglect among Emergency Medical Professionals in Pune.

Methodology: As part of an awareness initiative by the Institute, an online session was conducted for the Emergency Medical Professionals (EMP) in Pune city. 65 Emergency Medical Professionals working in various hospitals located around all parts of Pune participated in the session. After the session was completed, informed consent was taken from the participants for answering a questionnaire based on the subject.

The questionnaire chosen for the study was Child Abuse and Neglect Awareness Scale (CANA-S) a pretested and validated tool. The CANA-S tool comprises 4 sections, each section comprising a subscale dedicated to one of the major categories of child abuse and neglect i.e. physical abuse, sexual abuse, emotional abuse, and neglect.

Conclusion: Though the overall awareness about Child Abuse and Neglect is satisfactory, further training programs should be encouraged, to achieve near 100 % awareness amongst EMS professionals in order to prevent and report every single case of Child Abuse and Neglect.

Keywords: Child abuse, awareness, sexual abuse

Introduction

Violence against children is widespread and affects millions of children globally. Violence against children occurs in various forms including physical violence, maltreatment, bullying, emotional or psychological violence, forced and bonded labour, and trafficking.¹

Shockingly even children of the tender age of 2 - 4 years are not spared. 3 in 4 children in this age group are inflicted with physical punishment and/or psychological violence at the hands of parents and caregivers. This apart, as many as 1 in 5 women and 1 in 13 men report...
having been sexually abused as a child.  

India is home to over 19 percent of the world’s children, making it the country with the largest child population in the world. Hence the responsibility of our country in setting an example for child safety and abuse prevention is paramount.  

To prevent child abuse, it is essential that a high degree of awareness is essential amongst all stakeholders including parents, educators and medical professionals. A study by Ghanem E. et al (2015) meant to assess awareness of Child abuse among Egyptian Medical Students concluded that the majority of the respondents encountered cases of child abuse but their knowledge of child maltreatment still remains insufficient. 

Closer to home, a study conducted in Bagalkot district of North Karnataka by Kirankumar et al (2011) showed the glaring lack of knowledge accompanied with poor attitude and perception about Child Abuse Neglect. 

This was further reiterated in a study by Deshpande et al (2015) conducted in the state of Gujarat who assessed knowledge and attitude in regards to physical child abuse amongst medical and dental residents. The study signifies the gaps between recognizing signs of physical child abuse and responding effectively to it. 

The common thread among all studies conducted in medical professionals is the lack of focused training and sensitisation that can enhance the ability to detect such cases and respond to them promptly. 

Among all medical professionals, Emergency Medical Services (EMS) providers would most frequently encounter potential child abuse situations. The duty of EMS professionals is not restricted in only providing emergency medical care but extends to reporting the suspicion to the appropriate authorities in order to ensure child safety and prevent further abuse. 

A systematic review conducted by Johnson et al emphasized the importance of targeted training programs among EMS professionals to improve their ability to identify and report child abuse and neglect. 

The first step towards designing an effective training program is to assess the baseline awareness of Child Abuse and Neglect amongst EMS professionals. 

**Objective**

To Assess Awareness Regarding Child Abuse and Neglect among Emergency Medical Professionals in Pune

**Methodology**

As part of an awareness initiative by the Institute, an online session was conducted for the Emergency Medical Professionals (EMP) in Pune city. 65 Emergency Medical Professionals working in various hospitals located around all parts of Pune participated in the session. The session comprehensively covered subject of Abuse, Neglect and Assault with focus on identification of cases, legal aspects, roles and responsibilities of medical professionals, measures of intervention and management. It was an interactive session and the participants were given ample opportunity to share their views and clear their doubts on the subject. The information pertaining to the subject was shared through Power point presentation and the session was conducted by Medical Officer with five years of experience. The session was conducted via Microsoft Teams application for two hours and the participants interacted verbally, by unmuting themselves and messaging their views, doubts and inputs via chat in the Microsoft Teams application. 

After the session was completed, informed consent was taken from the participants for answering a questionnaire based on the subject. The questionnaire chosen for the study was Child Abuse and Neglect Awareness Scale (CANA-S) a pretested and validated tool, developed by Altan et al (2017). The reliability of the questionnaire is found to be high (Cronbach’s alpha value 0.768) for evaluating awareness about child abuse and neglect among the medical students. 

The CANA-S tool comprises 4 sections, each section comprising a subscale dedicated to one of the major categories of child abuse and neglect i.e. physical abuse, sexual abuse, emotional abuse, and neglect. Each section consists of 5 vignettes which requires response on a five-point Likert scale ranging from 5 to 1 as “definitely appropriate=5,” “appropriate= 4,” “uncertain=3,” “inappropriate=2,” and “definitely inappropriate=1”.
The highest and lowest possible scores for the full scale are 100 (indicating the highest level of awareness) and 20 (indicating the lowest level of awareness), respectively. All 20 items were retained and required to be mandatorily filled. The tool was administered through online mode. The respondents were given one-day deadline to revert with responses. Any queries pertaining to the tool questionnaire were clarified during data collection. 62 respondents reverted with completely filled questionnaire. The data was tabulated and statistically analysed with the help of SPSS version 23.

### Result

**Demographic Data:**

<table>
<thead>
<tr>
<th>Students</th>
<th>62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>49 (79.03 %)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>13 (20.96 %)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>51 (82.25 %)</td>
</tr>
<tr>
<td>Male</td>
<td>11 (17.74 %)</td>
</tr>
<tr>
<td>Qualification</td>
<td></td>
</tr>
<tr>
<td>MBBS</td>
<td>1</td>
</tr>
<tr>
<td>BHMS</td>
<td>29</td>
</tr>
<tr>
<td>BAMS</td>
<td>29</td>
</tr>
<tr>
<td>BUMS</td>
<td>2</td>
</tr>
<tr>
<td>BDS</td>
<td>1</td>
</tr>
</tbody>
</table>

The total scores were calculated for all the 20 items of the tool. The mean of Total score for all respondents is 70.49 with standard deviation of 7.59. The highest total score is 88 and the lowest is 50.
As shown in Figure 1, the mean awareness score for Physical and Emotional abuse is much lower as compared to Sexual Abuse and Neglect.

**PHYSICAL ABUSE:**

**EMOTIONAL ABUSE:**
As shown in Figures 2 & 3, the participants showed relatively lower awareness levels in 2 of the 5 vignettes based on both physical and emotional abuse.

**Sexual Abuse:**

![Sexual Abuse Mean Score](image)

As shown in Figures 4 & 5, the vignettes based on sexual abuse and neglect were responded with high degrees of awareness.

**Neglect:**

![Neglect Mean Score](image)
Discussion

The study was aimed to gauge the awareness levels of child abuse and neglect amongst EMP. The mean total score of 70.49 out of 100 is encouraging and reflects the fairly good degree of awareness. The sample included a majority of married female EMP’s which could partly be responsible for the higher degree of sensitivity on the subject. Even though the overall score is high there is a clear distinction in the level of awareness on the individual subscale. Due to greater emphasis on sexual abuse and neglect, both in the curriculum and the media; the understanding of the sign is better.

The results point towards greater focus on the presentations of cases of physical and emotional abuse in training programs. Sensitization programs and role play workshop can play a significant role in raising the knowledge and improving the attitude towards child abuse and neglect. Similar vignettes can be used to coach EMS professionals in correct identification and response in suspected cases of Abuse and Neglect.

The study is limited by its concise sample size but provides significant perspective on this longed ignored issue. Larger studies can further study the correlation of Sex and Marital Status on the degree of awareness of Abuse.

Conclusion

Though the overall awareness about Child Abuse and Neglect is satisfactory, further training programs should be encouraged, to achieve near 100% awareness amongst EMS professionals in order to prevent and report every single case of Child Abuse and Neglect.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Obtained from IEC, SIU

References

To Assess Preparedness in Disaster Management among EMS Professionals in Pune, India

Parag Rishipathak¹, Monesh Bhandari², Anand Hinduja³

¹Professor & Director, Symbiosis Centre for Health Skills, Symbiosis International (Deemed University), Pune, India, ²Medical Officer, Academics, Symbiosis Centre for Health Skills, Symbiosis International (Deemed University), Pune, India, ³Adjunct Faculty, Symbiosis Centre for Health Skills, Symbiosis International (Deemed University), Pune, India

Abstract

Introduction: Natural disasters cause an estimated 60,000 deaths every year. Although low-frequency, high-impact events like earthquakes and tsunamis are not preventable, albeit loss of human lives can be prevented by developing an efficient disaster management system. In the last few decades robust emergency response systems, emergency preparedness, resilient infrastructure and accurate forecasting have significantly reduced the death rate caused due to such calamities. Similar to natural disasters, MCIs drastically impact the healthcare system and society at large.

Emergency medical services (EMS) professionals play a pivotal role during disasters, mass casualty incidents etc. and are an integral part of disaster management apparatus. The level of awareness and preparedness amongst EMPs with regards to disaster management is primordial. The areas requiring improvement include skillset training strategic and operational planning and familiarity with the emergency preparedness in disaster management. Designing an effective training program for Indian EMS professionals, would require a thorough understanding of the baseline level of preparedness among them.

Objective: To assess preparedness in disaster management among Emergency Medical Professionals.

Methodology: The study was conducted among Emergency Medical Professionals (EMPs) employed in various hospitals in the city of Pune, India. The questionnaire tool utilized for the study was Emergency Preparedness Information Questionnaire (EPIQ) a pretested and validated tool. The revised EPIQ tool comprises of 42 items and is divided into eight sections.

All 42 items were required to be mandatorily filled. The tool was administered through online mode. 119 respondents reverted with completely filled questionnaire.

Conclusion: Disasters although infrequent, cause significant morbidity and mortality. A high degree of preparedness is expected amongst EMP’s which can only be achieved with regular training and feedback.

Keywords: Disaster, preparedness, emergency medical professionals, mass casualty

Introduction

Natural disasters cause an estimated 60,000 deaths every year. Although low-frequency, high-impact events like earthquakes and tsunamis are not preventable, albeit loss of human lives can be prevented by developing an efficient disaster management system. In the last few decades robust emergency response systems, emergency preparedness, resilient infrastructure and accurate forecasting have significantly reduced the death rate caused due to such calamities.¹
Mass casualty incidents (MCIs) defined as “events which generate more patients at one time, than locally available resources can manage using routine procedures” are increasing in frequency across the globe. Similar to natural disasters, MCIs drastically impact the healthcare system and society at large.\(^{2}\)

Emergency medical services (EMS) professionals play a pivotal role during disasters, mass casualty incidents etc. and are an integral part of disaster management apparatus.\(^{3}\) The role of EMS professionals in providing emergent healthcare services and upgrading the skill sets of the allied healthcare providers has been highlighted in a study by Catlett C.L \textit{et al} (2011).\(^{4}\)

Emergency Medical Professionals (EMP’s) can mitigate the damage caused by disasters only if they possess optimum levels of preparedness. Unfortunately, studies in the past have shown that the level of awareness and preparedness amongst EMPs with regards to disaster management is primordial. The areas requiring improvement include skillset training strategic and operational planning and familiarity with the emergency preparedness in disaster management.\(^{5,6}\) Regular training of EMS professionals, in disaster management is a need of the hour. Designing an effective training program for Indian EMS professionals, would require a thorough understanding of the baseline level of preparedness among them.

**Objective**

To assess preparedness in disaster management among Emergency Medical Professionals.

**Methodology**

The study was conducted among Emergency Medical Professionals (EMPs) employed in various hospitals in the city of Pune, India. The questionnaire tool utilized for the study was Emergency Preparedness Information Questionnaire (EPIQ) a pretested and validated tool, developed by Wisniewski \textit{et al} (2004)\(^{7}\) and later revised by Garbutt S.J. \textit{et al} (2008)\(^{8}\). The reliability of the questionnaire is found to be high (Cronbach’s alpha value 0.97) for evaluating awareness about disaster preparedness among the healthcare professionals.

The revised EPIQ tool comprises of 42 items and is divided into eight sections viz., (i) Incident command system factor (eight items) (ii) Triage factor (five items), (iii) Communication and connectivity factor (six items), (iv) Psychological issues and special populations factor (six items), (v) Isolation, decontamination, and quarantine factor (five items), (vi) Epidemiology and clinical decision-making factor (four items), (vii) Reporting and accessing critical resources factor (four items), (viii) Biological agents factor (four items). Each item requires response on a five-point Likert scale ranging from scores of 5 to 1 wherein 5 stands for “Very Familiar”, 4 stands for “Somewhat Familiar”, 3 stands for “Familiar to Neutral”, 2 stands for “Somewhat Unfamiliar” and 1 stands for “Not Familiar”. Informed consent was taken from the participants for answering the questionnaire based on the subject

All 42 items were required to be mandatorily filled. The tool was administered through online mode. The respondents were given one-day deadline to revert with responses. Any queries pertaining to the questionnaire were clarified during data collection. 119 respondents reverted with completely filled questionnaire. The data was tabulated and statistically analysed with the help of SPSS version 23.

**Result**

**Demographic Data**

<table>
<thead>
<tr>
<th>Participants</th>
<th>119</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>21 to 25 years</td>
<td>102 (85.71%)</td>
</tr>
</tbody>
</table>
Cont.. Table 1:

<table>
<thead>
<tr>
<th>Experience</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>26 to 30 years</td>
<td>11 (9.24%)</td>
<td></td>
</tr>
<tr>
<td>&gt;30 years</td>
<td>6 (5.04%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year to 3 years</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td>&gt;3 years</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>74 (62.7%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 (37.3%)</td>
<td></td>
</tr>
<tr>
<td>Qualification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBBS</td>
<td>1 (0.9%)</td>
<td></td>
</tr>
<tr>
<td>BHMS</td>
<td>54 (44.9%)</td>
<td></td>
</tr>
<tr>
<td>BAMS</td>
<td>37 (31.4%)</td>
<td></td>
</tr>
<tr>
<td>BUMS</td>
<td>24 (20.3%)</td>
<td></td>
</tr>
<tr>
<td>BNYS</td>
<td>3 (2.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Mean Familiarity Score

Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean Familiarity Index (Out of 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident command system factor</td>
<td>3.39</td>
</tr>
<tr>
<td>Triage factor</td>
<td>3.78</td>
</tr>
<tr>
<td>Communication and connectivity factor</td>
<td>3.32</td>
</tr>
<tr>
<td>Psychological issues and special populations factor</td>
<td>3.61</td>
</tr>
<tr>
<td>Isolation, decontamination, and quarantine factor</td>
<td>3.75</td>
</tr>
<tr>
<td>Epidemiology and clinical decision-making factor</td>
<td>3.43</td>
</tr>
<tr>
<td>Reporting and accessing critical resources factor</td>
<td>3.48</td>
</tr>
<tr>
<td>Biological agents factor</td>
<td>3.67</td>
</tr>
</tbody>
</table>

As seen in the Table 2, the familiarity across all parameters range between 3 to 4 indicating moderate degree of familiarity with all the aspects of Disaster Management
As seen in Figure 1 the most common response across parameters was found to be, ‘Somewhat Familiar’

Similarly, as shown in Figure 2 EMP’s were ‘Somewhat Familiar’ with the parameters regarding Disaster Management

Significantly as many as 6 to 10 % of the respondents, inspite of being working EMP’s had no familiarity with any disaster management protocol. Approximately, 10 to 14 % of the respondents had some vague idea but displayed lack of preparedness across all parameters of disaster management.

Discussion

The study was conducted with an aim to assess the degree of preparedness with regards to natural disasters and mass casualty incidents (MCI) among EMP’s. The results are somewhat encouraging as majority of the respondents displayed some degree of familiarity with the all-round aspect of disaster management.
It is crucial for working EMP’s to possess a high degree of knowledge and skill set with regards to disaster management as they are first responders. The results therefore highlight the emergent need of continuing medical education (CME) and practical workshops to equip EMP’s with the required skill set and knowledge. Although Disaster management is covered as a part of EMS curricula, periodic re–enforcement is essential to maintain optimum level of preparedness among EMP’s. Pre and post testing during disaster management workshops can help in objectively identifying the degree of improvement in preparedness and the effectiveness of the practical sessions.

**Conclusion**

Disasters although infrequent, cause significant morbidity and mortality. The role of EMP’s in positively impacting lives during disasters cannot be overemphasized. A high degree of preparedness is expected amongst EMP’s which can only be achieved with regular training and feedback.

**Conflict of Interest:** None

**Source of Funding:** Self

**Ethical Clearance:** Obtained from IEC, SIU

**References**


A Comparative Study between Nineveh and Tikrit Medical School Teachers Perceptions regarding the Effectiveness of OSCE in Clinical Examination

Wafa Mahmood Jasim¹, Zahraa Ahmed Hassan²

¹Lecturer, Family & Community Medicine / Northern technical university / Kirkuk Technical Institute, ²Community Medicine/ Collage of Medicine / Kirkuk University, Researcher

Abstract

Assessment students competence is of much importance especially when evaluation the expected learning out come of medical education and because of increasing students number enrolled in medical colleges, this lead to enhance the chance of malpractice that compromise patient conditions, therefore it is challenging to develop and implement such an objective assessment method in clinical examination. Objective of the study: The study aim is to compare the traditional and innovative medical school teachers perceptions regarding the effectiveness of OSCE in clinical examination. A descriptive cross sectional study has been conducted in both (Nineveh and Tikrit medical college from the period of 15th February till 20 th April / 2014. The study was included 95 medical teachers from both traditional and innovative schools (55 teachers from the traditional school and 40 from innovative school). A special questionnaire form was prepared by the researcher through direct interviewing with the study sample. The results show that 63.1% of medical teachers are male, 49.5% aged between 40-49 years, 65.3% from basic specialty sciences, 51.6% having a period more than 10 years in teaching, 70.9% of traditional school teachers agree that OSCE evaluate the practical objectives in comparison to 67.5% of innovative school teachers agree with that OSCE evaluate knowledge, understanding, practical, and intellectual objectives. The study concluded that more than half of traditional medical teachers agree with the affectivity of OSCE in assessing large number of students without bias while medical teachers from innovative school agree with the objectivity of OSCE in clinical evaluation.

Key words :- Medical teachers, Perceptions, clinical examination, Kirkuk, Iraq.

Introduction

Assessment usually referred to as a evaluation system for all professional accomplishments by using a different defined criteria which mainly having an attempt at a measurement point either by assigning numerical value or grading on a rough scale. Assessment of medical students skills and their knowledge is of necessary benefits because it is not only filters the best students but continuous monitoring which leads for better future physician. A specific documented assessment test should produce the similar scores for two or more situations occasions or if corrected by two or more examiners. The validity of a test is determined by the extent to which it measures whatever it sets out to measure.

Usually many different methods of medical students assessment such as multiple choice questions (MCQ), modified assay questions (MAQ), viva voce (VV), long case discussion and lastly objective structured clinical examination (OSCE).

Each one of these evaluation method may express the psychomotor, cognitive or students behavioral skill but rarely it can prudent all these skills in one single method but the most best one of these that encounter the
indicative purpose is OSCE where the medical students face a standardized patient prepared for examination purpose in front of the examiner. OSCE was first mentioned in clinical examination by Harden since 1975 and during this period OSCE was suitable for both of students and faculty.

OSCE is mainly used for evaluation of basic and clinical skills. Students are assessed at different a number of “stations” on discrete focused activities that simulate many parts of clinical competence. For each station a standardized patients (SPs), real patients or simulators may be used and full explained demonstration of each specific skills can be observed, monitored and measured. OSCE stations also have the ability to incorporate the assessment of non-patient skills, technical skills, and interpretation.

Although of a complete comprehensive coverage of the whole curriculum that is possible to done, OSCE has one disadvantage through losing the real aspect to a patient due to segregated encounters at many different station of it. In order to obtain a specific measurable test of performance reliability is a function of sampling, it should have a number of competences tested and stations. So for calculating the score with a task specific checklist or a combination of a rating scale and checklist together.

The aim of the study is to compare the traditional and innovative medical school teachers perceptions regarding the effectiveness of OSCE in clinical examination.

Subjects and methods:

Sampling methods:

A cross-sectional study was done among medical teachers in Nineveh and Tikrit medical college and 95 teachers from both basic and clinical departments were included in the study (55 teachers from traditional school and 40 from innovative school).

A certain specific questionnaire sheet was distributed to all participants after taking their written agreements and the data was obtained through face to face method after full brief explanation of the study aim.

Study period and setting:

The study was preformed during the period from 15th February until 20th April/2014 according to special time table which has been prepared by the investigator in both Nineveh and Tikrit college of medicine.

Tool for obtaining the data:

A certain questionnaire sheet has been designed by the investigator contain the following parts:

Part-1- Demographic characteristics including (age, gender, certificate, period in teaching, specialty and scientific degree) which has been taken from the unite of human resources at each medical collage.

Part-2- Teachers perceptions about the main objectives evaluated by OSCE.

Part-3- Teachers perceptions about the affectivity of OSCE in clinical examination.

Part-4- Teachers perceptions regarding the factors affecting the application of OSCE.

Part-5- Teachers suggestions for future high quality OSCE clinical examination.

Ethical issues:

Agreements were obtained from both Nineveh and Tikrit college of medicine before starting the research.

Analysis of the data statistically:-

For the statements contain yes and no answer, number and % was done.

The relation between the studied variables was done by calculating Chi-square test and (P < 0.05) was regarded significance at level 5%.

Results:

Table 1 show that 63.1% of medical teachers are male, 49.5% aged between 40-49 years, 65.3% from basic specialty sciences, 51.6% having a period more than 10 years in teaching, and 37.9% are lecturers. Table 2 show that 70.9% of traditional school teachers agree that OSCE evaluate knowledge, understanding, intellectual and practical objectives with p value = 0.000. Table 3 presents that 69.1% of
traditional medical teachers agreed with the affectivity of OSCE in assessing large number of students without bias while 80% of innovative medical teachers agree with the objectivity of OSCE in clinical evaluation in different specialties with a p value = 0.084

On the other hand both of traditional and innovative medical teachers disagree that OSCE is saving time and effort with a p value =0.073 . Table 4 shows that male teachers from both traditional and innovative schools go with the OSCE tasks and commands did not demonstrated correctly before exam (41.9% 51.7%) respectively with a p value =0.448. On the other hand 41.7% females from traditional schools agree that there was no adequate time for each station while 54.5% female from innovative schools agree that there was an interference during exam which disturb it with a p value =0.261 . Table 5 presents that traditional medical teachers from both basic and clinical departments suggested a combination of innovative and traditional assessment methods to get a better future results (43.6%, and 50.0%) respectively with a p value =0.593. For the innovative school, the medical teachers from basic departments suggested the necessity for more advanced training educational program for staff member to increase their ability about the correct application of OSCE (47.8%), while (58.9%) teachers from clinical departments suggested the support from college administration to implement and development of OSCE with a p value =0.045.

Table 1: Frequency distribution of medical schools teachers according to their socio demographic characteristics

<table>
<thead>
<tr>
<th>Socio demographic parameter</th>
<th>Traditional school (Nineveh) N=55</th>
<th>Innovative school (Tikrit) N =40</th>
<th>Total N= 95</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>29</td>
<td>60</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>11</td>
<td>35</td>
</tr>
<tr>
<td>Age group (in years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30- 39</td>
<td>18</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>40- 49</td>
<td>20</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>50 -59</td>
<td>13</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Certificate</td>
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<td></td>
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</tr>
<tr>
<td>Master of science Board (Iraqi and Arabian) PhD</td>
<td>17</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>23</td>
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</tr>
<tr>
<td></td>
<td>23</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>Specialty</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic sciences</td>
<td>39</td>
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<td>62</td>
</tr>
<tr>
<td>Clinical sciences</td>
<td>16</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>Period in teaching</td>
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<td></td>
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</tr>
<tr>
<td>&lt; 5 years</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>5-10 years</td>
<td>19</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>&gt; 10 years</td>
<td>28</td>
<td>21</td>
<td>49</td>
</tr>
<tr>
<td>Scientific degree</td>
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<tr>
<td>Professor</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Assistant professor</td>
<td>12</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Lecturer</td>
<td>21</td>
<td>15</td>
<td>36</td>
</tr>
</tbody>
</table>
Table 2: Distribution of study medical teachers according to their perceptions regarding the types of objectives evaluated by OSCE in clinical examination

<table>
<thead>
<tr>
<th>Types of objectives evaluated by OSCE</th>
<th>Teachers perceptions</th>
<th>Innovative school (Tikrit) N=40</th>
<th>P* Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional schools (Nineveh) N=55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agree</td>
<td>Disagree</td>
<td>Agree</td>
<td>Disagree</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Knowledge, understanding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>54.5</td>
<td>25</td>
<td>45.5</td>
</tr>
<tr>
<td>Intellectual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>67.3</td>
<td>18</td>
<td>32.7</td>
</tr>
<tr>
<td>Practical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>70.9</td>
<td>16</td>
<td>29.1</td>
</tr>
<tr>
<td>All the above</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>30.9</td>
<td>38</td>
<td>69.1</td>
</tr>
</tbody>
</table>

* $\chi^2$ – test was used

Table 3: Distribution of study medical teachers according to their perceptions regarding the affectivity of OSCE in clinical examination

<table>
<thead>
<tr>
<th>Affectivity of OSCE in clinical examination</th>
<th>Teachers perceptions</th>
<th>Innovative schools (Tikrit) N=40</th>
<th>P* Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agree</td>
<td>Disagree</td>
<td>Agree</td>
<td>Disagree</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>1- Improve students performance by setting clinical scenarios</td>
<td>29</td>
<td>52.7</td>
<td>26</td>
</tr>
<tr>
<td>2- Objectivity in clinical evaluation in different specialties</td>
<td>35</td>
<td>63.6</td>
<td>20</td>
</tr>
<tr>
<td>3- Valid and reliable method of evaluation</td>
<td>22</td>
<td>54.6</td>
<td>27</td>
</tr>
<tr>
<td>4- Save time and effort</td>
<td>15</td>
<td>27.3</td>
<td>40</td>
</tr>
<tr>
<td>5- Assessing large number of students effectively without bias</td>
<td>38</td>
<td>69.1</td>
<td>17</td>
</tr>
</tbody>
</table>

$\chi^2$ – test was used
Table 4: Distribution of medical teachers according to factors affecting OSCE in clinical examination

<table>
<thead>
<tr>
<th>Factors affecting OSCE</th>
<th>Medical teachers perceptions</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional schools (Nineveh)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N= 55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>1- Interference during examination</td>
<td>4 12.9</td>
<td>2 8.3</td>
<td>7 24.1</td>
<td>6 54.5</td>
<td>0.261</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2- OSCE tasks and commands did not demonstrated correctly before exam</td>
<td>13 41.9</td>
<td>5 20.8</td>
<td>15 51.7</td>
<td>3 27.3</td>
<td>0.448</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3- Inadequate time for each OSCE station</td>
<td>7 22.5</td>
<td>10 41.7</td>
<td>4 13.8</td>
<td>1 9.1</td>
<td>0.379</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4- Bed side objective clinical teaching is not covered in OSCE exam</td>
<td>5 16.2</td>
<td>4 16.7</td>
<td>3 10.4</td>
<td>1 9.1</td>
<td>0.510</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5- Facilitator is not cooperative</td>
<td>2 6.5</td>
<td>3 12.5</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>24</td>
<td>29</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2$ – test was used
Table 5: Distribution of medical teachers according to their perceptions regarding future suggestions for high quality OSCE in clinical exam

<table>
<thead>
<tr>
<th>Future suggestions for high quality OSCE in clinical exam</th>
<th>Medical teachers perceptions</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P* Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional schools (Nineveh)</td>
<td>Innovative school (Tikrit)</td>
<td>N= 55</td>
<td>N=40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basic</td>
<td>Clinical</td>
<td>Basic</td>
<td>Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>1- College administration support</td>
<td>11</td>
<td>28.2</td>
<td>4</td>
<td>25.0</td>
<td>6</td>
<td>26.1</td>
</tr>
<tr>
<td>2- Adequate resources</td>
<td>6</td>
<td>15.4</td>
<td>2</td>
<td>12.5</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>3- Advanced training educational program for teaching staff</td>
<td>5</td>
<td>12.8</td>
<td>2</td>
<td>12.5</td>
<td>11</td>
<td>47.8</td>
</tr>
<tr>
<td>4- Combination of both traditional and innovative assessment methods</td>
<td>17</td>
<td>43.6</td>
<td>8</td>
<td>50.0</td>
<td>4</td>
<td>17.4</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>16</td>
<td>23</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ² test was used

Discussion

The current study show that traditional school teachers agree with the OSCE evaluate the practical objectives in comparison to innovative school teachers who agree with that OSCE evaluate knowledge, understanding, practical, and intellectual objectives.

A study was conducted by 13. In Qassim university / King fahad specialists hospital to evaluate the reliability and validity of the interactive OSCE in the medicine and to evaluate the appropriate time for each station. They found that 84% of the external and internal examiner that OSCE satisfy covering the whole skills needed by the junior physician who well work under supervision but they reported that the duration of each station can be increased to get a better results during the examination.

Similar results were obtained by 14, 15. and Siddiqui 16. that OSCE is a very reliable and valid performance test through using an evaluating sheet and it is a satisfactory method in addressing the intended purposes which were designed for it if correctly applied.
Regarding the affectivity of OSCE in clinical examination, the present study revealed that traditional medical teachers agree with the affectivity of OSCE in assessing a large number of students without bias while innovative medical teachers agree with the objectivity of OSCE in clinical evaluation in different specialties.

A study was done by Alaa et al in Tikrit university / medical college / 2013\textsuperscript{17} to overcome rater variation in clinical assessment of medical students through improving the use of OSCE as an assessment tool in Tikrit university / College of medicine.

They found that OSCE is used by 30% for assessment at the end of their clinical clerkship for 6\textsuperscript{th} year study only and there was no suitable comfortable place for OSCE application and only 40% of faculty members were interested in development and implementation of OSCE in clinical evaluation.

They mentioned that the main obstacles and root causes of OSCE development are (some faculty and students don’t know what is OSCE, its validity and reliability in addition to that some staff members are not adequate trained about how to implement OSCE).

The current study show male teachers from both traditional and innovative schools go with that the main factor affecting OSCE is the tasks and commands did not demonstrated correctly before exam.

A study done by \textsuperscript{18} in Cairo and Ain Shams university / Egypt to build a capacity of nursing faculties and staff member for OSCE establishing simulated learning experience in clinical practice and comparing the feasibility, utility and effectiveness of using OSCE as an assessment method with the comparison between students and faculties perceptions for OSCE validity and reliability. They reported from their study that 57% of faculty member knew nothing about OSCE and 98% of them had no experience previously in using OSCE.

They concluded that OSCE provide an attractive options for students assessment competency and giving a practical strength points for faculty staff objectivity and reliability for all students assessment especially when OSCE compared with traditional practical assessment methods and the most important factor attributing for OSCE evaluation is the adequate time for each station.

Concerning the future suggestions for better OSCE, the current study show that traditional medical teachers from both basic and clinical departments suggested a combination of innovative and traditional assessment methods.

This result is agree with a study done by \textsuperscript{19} 2008 about the OSCE and its reliability and validity in medical students evaluation with a review of the consequences of OSCE method and outlines the important issues for medical educators in order to consider when there is a need for its application in the educational program in the future. They found that medical educators usually needed the most useful reliable valid method for both formative and summative students evaluation because such method is regarded as a useful for learner, faculty, institutional, and for the public at large are great.

They mentioned that OSCE has become the standardized practice in modern assessment of students clinical competence and the outcomes for this test are generally used for high – stakes decision making at different levels, in addition to that, they said that the correct suitable planning coordination of multiple resources, commitment to large scale testing in order to get a significant results.

**Limitation of the study:**

Difficulty in collecting the questioners sheet with the over load students schedule

**Conclusions**

1- OSCE evaluate the knowledge, understanding, intellectual and practical objectives.

2- Affectivity of OSCE through assessing a large number of students without bias and the objectivity of OSCE in clinical evaluation in different specialties.

3- There is a need for OSCE tasks and commands to be demonstrated correctly before exam.

4- Adequate time is necessary and avoid interference during exam.

**Acknowledgment:** My special thanks to all the staff for both Nineveh and Tikrit medical college and all the students who participated in the study.
Ethical issues: Agreements were obtained from both Nineveh and Tikrit college of medicine before starting the research.

Source of Funding: Self

Conflict of Interest: Nil

References


Assessment of Nurses Knowledge about Alzheimer’s disease

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¹Assistant Lecturer, Adult Nursing Department, College of Nursing/University of Thi-Qar, Iraq

Abstract

Insufficient information regarding Alzheimer’s disease (AD) among the healthcare workers can harmfully affect outcomes of the patient. This designate the necessity for constant knowledge supplement on AD among them in order to deliver the best care to AD patients as possible and propose the necessity to evaluate the level of AD understanding among nurses and identify their shortages so as to improve care quality and promote life quality for individuals with AD. Aims of the study: Current study designed to (i) evaluate the knowledge concerning AD among the nurses working in the hospitals of Thi-Qar governorate/Iraq, and (ii) measure the relationship between selected nurses’ demographics and their knowledge on AD. A survey design was applied to assess the nurses’ knowledge regarding AD.

(i) Self-designed questionnaire was used to collect Socio-demographic characteristics of the participants which included of 7 items (age, gender, years of experience, educational level, department of work, previous training, and previous caregiving experience).

(ii) Alzheimer’s Disease Knowledge Scale (ADKS) were used to assess nurses’ knowledge regarding AD. This study was conducted in four Teaching Hospitals in Thi-Qar governorate/Iraq. A 622 nurses were participated in the study. The results involved answers from 622 nurses. Nurses knowledge was in average (Mean=15.93). Also, there were a statistically significant relationship between certain demographic characteristics (Age, previous training, and previous caregiving experience) and knowledge of the nurses concerning AD. This study exhibited many findings on this important subject, in addition to the demographic characteristics and its relation to nurses’ knowledge.

Keywords: Nurses, knowledge, Alzheimer’s disease, Iraq

Introduction

The incidence of AD has escalated concerning the rapid rising of elderly population, thus reveal major challenges to delivery of care for patients with AD.

AD is a progressive and chronic disease with an average period of 7 to 10 years. Adults having AD spend more duration in the final phase, including severe dementia, compared with previous moderate and mild phases (¹) current approximations set the prevalence of individuals with AD at just under 5 million where almost every 60 seconds someone in the United States develops AD (²) and predict that until 2050 approximately 14 million individuals will match the diagnostic standards in the United States only (³).

This will impose challenges to healthcare professionals that is already scarce in number and preparation. Moreover, staff preparation must start with understanding the diverse requirements of care that are specified for the 3 phases of AD (initial, central, and final).

In contrast to individuals with other chronic diseases, older people looking for aid for AD are usually got the consideration of a healthcare professional through their relatives. Pursuing a diagnosis of AD may be difficult, as the individuals having the illness may not own understandings concerning their symptoms. Furthermore, since there is a stigma linked to AD,
families might be unwilling to look for a diagnosis or assistance for their patients (4).

While the cognitive and practical restrictions of an individual having AD rise, long-term care and support are essential to help them with daily responsibilities for instance cleaning, wearing clothes, and taking medications. Around 87% of people needs long-term care, many of individuals having AD, obtain these services by unpaid relative care providers (5,6).

Finally, once cognitive deterioration develops and brain injury turns more aggressive, people having AD go through a sharp physical deterioration and need concentrated support. Certain families can be able to assist a relative having final-phase AD in their houses. Still, many individuals will spend a period in medical institutions (7).

Insufficient information regarding Alzheimer’s disease (AD) among the healthcare workers can harmfully affect outcomes of the patient. This designate the necessity for constant knowledge supplement on AD among them in order to deliver the best care to AD patients as possible (8). And it crucial to evaluate the knowledge level of AD among staff nurses and report their knowledge gaps with the purpose of improve the care quality and enhance life quality for AD patients (7).

Literature evidence collected from developing countries is limited because of poor AD services and scarce studies that were carried out to evaluate healthcare personal knowledge on AD (9). Consequently, current study designed to (i) evaluate the knowledge concerning AD among the nurses working in the hospitals of Thi-Qar governorate/Iraq, and (ii) measure the relationship between selected nurses’ demographics and their knowledge on AD.

Methods -Setting and Participants of the Study

Descriptive (survey design) was used during the period from 9th of September 2019 to 21st of February 2020 in four teaching hospitals in Thi-Qar governorate/Iraq. A random sample of 640 nurses were requested to participate in the study with a response rate of ≈97% (622 nurses). The sample was selected through the use of simple random sampling technique.

The Study Instrument:

Self-administrated questionnaire was used to collect the data, the questionnaire consists of two parts:

(i) Self-designed form was used to gather Socio-demographic characteristics of the participants, it composed of 7 items (age, gender, years of experience, educational level, department of work, previous training, and previous caregiving experience).

(ii) The tool that used to measure nurse’s knowledge was Arabic version of Alzheimer’s Disease Knowledge Scale (ADKS), as its easy to use, proven validity and reliability, and feasibility for variety of participants. It includes 30 false/true items with the end score is the items number that correctly answered (Example items: (i) People with AD are mainly susceptible to depression, (ii) mental exercise is scientifically proven to prevent a person from getting AD, (iii) After the appearance of AD symptoms, the average life expectancy is 6 to 12 years). Tool reliability was calculated through test-retest correlation = .81. The ADKS is theoretically divided into 7 content domains “risk factors, assessment and diagnosis, symptoms, course of disease, life impact, caregiving, and treatment and management” (10). The overall score was calculated by the addition of the correct responses to reach a score that ranges from 0 to 30.

- Pilot Study:

Pilot study was conducted on 30 nurses to test the validation of the questionnaire before the start of the study. The data from the pilot study were analyzed as the data of the main study. The internal consistency of the questionnaire for the study variables was performed by applying Cronbach’s alpha test (α = 0.82).

- Data Analysis:

With the purpose of analyze the collected data, the statistical package of social sciences (SPSS) ver. (23) were used. For descriptive statistics, percentage, frequency, mean, and standard deviation (SD) were used to analyze demographics and ADKS scores. The predictor of ADKS score was determined using multiple linear regression.

- Ethical Considerations:

Ethical agreement was approved from the Committee.
of Ethics of Thi-Qar health directorate. Approval to use the ADKS was attained from their developers. A cover letter has been introduced along with the questionnaire that distributed to clarify the objectives and course of the study. Contribution in the present study was confidential and voluntary, in addition to an informed consent that was attained from every participant before participating in the current study.

**Results**

-Socio – demographic variables:

Out of the (640) questionnaires distributed, 622 questionnaires were returned (response rate = 97). The mainstream of respondents were females (63.3%) and 34.2% were aged between 18 and 25 years (mean = 23.5 years, SD = 2.6), and over a half of them (55.8%) had 1-5 years of experience at work. The vast majority of participant (89.1%) reported no previous training concerning AD, and (71.9%) stated no previous caregiving experience for patients with AD. Other demographical features of the participants are mentioned in Table 1.

<table>
<thead>
<tr>
<th>SDVs</th>
<th>Group</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td>622</td>
<td>100</td>
</tr>
<tr>
<td>Age</td>
<td>18-25 years</td>
<td>213</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td>25-29 years</td>
<td>188</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>30-34 years</td>
<td>149</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>35 years and over</td>
<td>72</td>
<td>11.6</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>228</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>394</td>
<td>63.3</td>
</tr>
<tr>
<td>Years of experience</td>
<td>1-5 years</td>
<td>347</td>
<td>55.8</td>
</tr>
<tr>
<td></td>
<td>6-10 years</td>
<td>123</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>11 years and over</td>
<td>152</td>
<td>24.4</td>
</tr>
<tr>
<td>Educational level</td>
<td>Nursing School</td>
<td>190</td>
<td>30.5</td>
</tr>
<tr>
<td></td>
<td>Diploma</td>
<td>203</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>Bachelor</td>
<td>199</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>Master and Higher</td>
<td>30</td>
<td>4.8</td>
</tr>
<tr>
<td>Department of work</td>
<td>General Wards</td>
<td>309</td>
<td>49.7</td>
</tr>
<tr>
<td></td>
<td>Emergency Department</td>
<td>125</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>Consultation Unit</td>
<td>103</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Psychiatric Wards</td>
<td>85</td>
<td>13.7</td>
</tr>
<tr>
<td>Previous training on AD</td>
<td>Yes</td>
<td>68</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>554</td>
<td>89.1</td>
</tr>
<tr>
<td>Caregiving experience</td>
<td>Yes</td>
<td>175</td>
<td>28.1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>447</td>
<td>71.9</td>
</tr>
</tbody>
</table>
- Percentage, N= number of sample.

**ADKS scores:**

The overall mean score of ADKS was 15.93 (SD = 0.87) out of 30 and equal to 56% of correct responses (Table 2). Domains with the lowest responses included those related to course of disease, risk factors, and caregiving (percent correct = 28.5%, 35.7%, and 36.4% respectively). While life impact and symptoms domains recorded the best responses with a correct response rate of 79.7 and 75.8 respectively.

<table>
<thead>
<tr>
<th>Domain</th>
<th>No. of Items</th>
<th>(Mean/SD) ADKS score</th>
<th>of</th>
<th>% of correct answers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factors</td>
<td>6</td>
<td>2.11/0.17</td>
<td></td>
<td>35.7</td>
</tr>
<tr>
<td>Assessment and diagnosis</td>
<td>4</td>
<td>2.55/0.92</td>
<td></td>
<td>63.8</td>
</tr>
<tr>
<td>Symptoms</td>
<td>4</td>
<td>3.03/1.07</td>
<td></td>
<td>75.8</td>
</tr>
<tr>
<td>Course of disease</td>
<td>4</td>
<td>1.14/1.03</td>
<td></td>
<td>28.5</td>
</tr>
<tr>
<td>Life impact</td>
<td>3</td>
<td>2.39/0.79</td>
<td></td>
<td>79.7</td>
</tr>
<tr>
<td>Caregiving</td>
<td>5</td>
<td>1.82/1.12</td>
<td></td>
<td>36.4</td>
</tr>
<tr>
<td>Treatment and management</td>
<td>4</td>
<td>2.89/1.02</td>
<td></td>
<td>72.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>15.93/0.87</strong></td>
<td></td>
<td><strong>56.02</strong></td>
</tr>
</tbody>
</table>

**Predictors for the ADKS scores:**

Predictors of significance to participants knowledge in this study were age and previous caregiving experience. Age was the factor of highest effect on overall knowledge ($\beta = -.27; p=.021$), followed by previous caregiving experience ($\beta = .12; p <.001$). Other variables show no significant relationship with overall knowledge, other details Table (3).
<table>
<thead>
<tr>
<th>Independent variables</th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>t value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>25.12</td>
<td>0.52</td>
<td></td>
<td>34.12</td>
<td>.000</td>
</tr>
<tr>
<td>Age</td>
<td>−1.45</td>
<td>0.43</td>
<td>−0.27</td>
<td>−3.58</td>
<td>.021*</td>
</tr>
<tr>
<td>Gender</td>
<td>0.14</td>
<td>0.02</td>
<td>0.13</td>
<td>2.23</td>
<td>.091</td>
</tr>
<tr>
<td>Years of Experience</td>
<td>−0.52</td>
<td>0.56</td>
<td>−0.07</td>
<td>−1.88</td>
<td>.080</td>
</tr>
<tr>
<td>Educational Level</td>
<td>−0.14</td>
<td>0.28</td>
<td>−0.04</td>
<td>−0.36</td>
<td>.623</td>
</tr>
<tr>
<td>Department of Work</td>
<td>1.03</td>
<td>0.28</td>
<td>1.81</td>
<td>0.05</td>
<td>.089</td>
</tr>
<tr>
<td>Previous Training</td>
<td>0.97</td>
<td>0.35</td>
<td>0.85</td>
<td>2.88</td>
<td>.321</td>
</tr>
<tr>
<td>Previous Caregiving Experience</td>
<td>0.75</td>
<td>0.30</td>
<td>0.12</td>
<td>2.54</td>
<td>.000*</td>
</tr>
</tbody>
</table>

B: unstandardized coefficients, SE: Std. error of B, β: standardized coefficients. *P-value is significant (<0.05).

**Discussion**

Caring for patient with AD is regarded as one of the trending issues in public health globally nowadays especially in developing countries (including Iraq), which somehow have a less developed healthcare systems. This study investigated nurses’ knowledge regarding AD in Al-Nasiriyah city/Iraq.

The findings of this study reveal different levels of knowledge concerning the study subject where certain ADKS domains (life impact and symptoms) score highest percentage of correct answers while other domains (course of disease, risk factors) score the lowest, which is agreed with other studies (11,12) and this proposes that AD knowledge and training activities is a chief concern that needs to be addressed in Iraq especially that handling the health of individuals with AD necessitates continuing education and training and particular knowledge in AD care for healthcare professionals participating in primary care (13,14).

AD knowledge among nurses should be a major concern in public health, nurses that work in hospitals must have wider understanding and knowledge of AD and the management methods and they should be trained to preserve the residual intellectual capacities of the patient, aid them to sustain the independence in carrying out their everyday activities and evade injuries and deliver better life quality (15,16).

AD management is considered effective when it based on a firm base knowledge including the physiology, pathology, psychology, pharmacological therapy and caregiving of AD among healthcare providers (17). Deficits are present across nearly all participants in certain parts of the assessment, specifically in the risk factors, course of the disease, and care giving domains. Our results closely resemble the findings from the previous studies (11,17,18).

Other findings of the current study reveal significant relationship between certain demographic variables
(age and previous caring experience). Age variable was of the highest effect on overall knowledge followed by previous caring experience variable, and multiple studies agrees with current findings \(^{(11,13,16)}\)

**Conclusion**

In the current study, the mainstream of the nurses has moderate ADKS score, and this indicates a necessity for better educational grounding in AD care. Also, Age and previous caregiving experience are the most effective factors on overall knowledge about AD which should be considered in production of a specifically-tailored programs that help increasing AD Knowledge among nurses.

Two main limitations were faced during the current study. The first one is that the staff population in the 4 hospitals may not reflect hospitals in other districts of Thi-Qar governate or other parts of Iraq when generalizing the findings. Second, True and false designed questionnaire (even when its validated) make it complex to comprehend the real knowledge level of AD. Tools that simulate case studies may be used as a more effective tool that may be used in future studies.

**Disclosure**

Authors declare no conflicts of interest in this study.

**Source of Funding:** Self

**Ethical Clearance:** Ethical clearance was obtained from Thi-Qar health directorate ethical committee to conduct this study. Also, all participants were provided with information sheet about the study background, objectives, and if they are willing to participate in the study by answering a simple yes or no question.

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Demographic and Clinicohistological Profiles of Women Diagnosed with Breast Cancer at Al-El Wiya Maternity Teaching Hospital / Baghdad: A Retrospective Study

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2Consultant General Surgeon Women tumor center at Al-El Wiya maternity teaching hospital/Baghdad / Iraq

Abstract

Knowing the incidence of, breast cancer, diagnosis, and treatment methods given a strategic approach for community awareness and rapid management. This study was aimed to: Estimate the demographic pattern including age, marital status, number of children, mass location, lactation, using contraceptive and smoking habit and to estimate the pattern of BC risk factors including family history, histological type, grading, and staging. The retrospective cross-sectional descriptive design was utilized. A total of 282 confirmed breast cancer female patients for the years 2016 to 2018 were included. Overall result showed that the age of Breast cancer female patients ranged from (25-80) years with a mean (49.12±0.67). Most Breast Cancer cases were diagnosed with infiltrating ductal carcinoma (73.4%). The percentage of the discovered tumor was dominating the right side of the breast (52.8%). Also, 11% of BC females were diagnosed with stage Ila. And 31.2 % of BC cases were diagnosed as grade II. However, 20.2% of the cases were having a family history with breast cancer. Most of the cases (94.7%) were did not checked for biomarker testing, just 12 cases (4.3%) were positive ER. And 3.9% of cases tested for PR only 11 cases were positive. While negative results for HER2/neu was only in 9 cases (3.2%). Current study validates scientific knowledge about BC in Baghdad. Each year, the incidence rates increase especially for age above 49.

Keywords: Iraqi breast cancer, awareness, early detection, biomarkers, retrospective, ductal carcinoma

Introduction

Cancer incidence looks like a scale of diatonic, the increasing faced by increasing in society age and unhealthy lifestyles and premature death that would reduce the country’s productivity. According to data published by the Globocan site that in the 2030 year about 13 million people may die from cancer incidence worldwide, three-quarter of them may be in low income and middle-income countries (1). The breast cancer new cases increasing gradually and stand at the top first rank among other cancer in Iraq depending to the Annual report of the Iraqi cancer registry and this affected the community population (2). The innovation of several genetic factors has been found to detect breast cancer for example; BRCA1 and BRCA2 were the most important tests, followed by MYC and P53. These tests are available, more specific

The Iraqi national program for early detection of breast cancer, which was initiated since 2001, in an attempt to down-stage this disease at the time of presentation. Since then, specialized centers and clinics for early detection of breast tumors have been established in the major hospitals in all Iraqi provinces. Most screening and biological detection techniques simplify the early diagnosis of breast cancer; immunohistochemistry for biomarkers detection (2), ELISA for blood assay analysis (3), and the golden standard method is the presence of screening programs using mammography as an imaging technique (4,5). Still, self-breast screening is the major easy early detection method (5). All these techniques were present in the Iraq strategy of screening programs.

The innovation of several genetic factors has been found to detect breast cancer for example; BRCA1 and BRCA2 were the most important tests, followed by MYC and P53. These tests are available, more specific
but very costly for in low come countries, as in Iraq (6). Then adventure of new assays for gene detection using mammmaprint and Oncotype DX (7) but still not very widely in use.

Pieces of information about the incidence rate of breast cancer are important in planning health issues. In Iraq, the respective nesses of the present project were largely unclear partly due to inconsistency in data collection, an outcome that may be linked to the absence of a true cancer registry. This study was aimed to:

1. Estimate the demographic pattern including age, marital status, number of children, mass location, lactation, using contraceptive and smoking habit

2. Estimate the pattern of BC risk factors including family history, histological type, grading, and staging.

3. Due to lack of biomarker data for almost patient data here not shown in the result. Assess the pattern of BC characteristics, including HER2/neu status, estrogen and progesterone receptor status, CA15-3, CEA, P53, and Ki-67 biomarkers.

**Methods**

A descriptive – retrospective study has been conducted by using an information system data base from Al-Alwyia / oncology unit/ breast cancer center/ Baghdad /Iraq. In order to obtain representative data of patients, these data were collected during women visiting the center for checkup and examination for any breast problems 2016, 2017, and 2018 years. A total of 282 patients were included during these years.

The patients were asked for several pieces of information and each patient has his own file according to the Iraqi Cancer Registry, the list of questions contains age, number of children, using the contraceptive, side of the mass, smoking habit, family history with breast cancer, marital status and lactation. Then the data of results from their consequence visiting the center were collected and included: mammography result, FNA test, type of tissue, histological result, TNM, the grade of tissue, BI-RADs, and the result of biomarkers (ER, PR, Her2/neu, P53, Ki-67, CEA, CA15-3, AFP, and Keratin).

**Statistical Analyses**

Statistical analyses were run using SPSS software for Windows (version 24; IBM SPSS). The variables that were significant in the univariate analysis were included in the multivariate model. The data were analyzed and the frequency and the percentage were calculated accordingly. All of the statistical tests were P<0.05 indicated a significant difference.

**Ethical statement**

The present study was approved by the Research Ethics Committee of the cancer research center and according to the ethical standards laid down by the declaration of Helsinki. The ethical committee was obtained from the Health Research Unite and protocol Review Committee in the Ministry of Health / Baghdad / Iraq.

**Results**

The demographic characteristics of the patients were presented in Table 1, and their age distribution was presented in figure- 1. The main clinical features and the histopathological features were shown in Tables 1, 2, and 3, respectively. In total, 282 cases of women diagnosed with breast cancer were included in this study from 2016-2018 years. The age at diagnosis of the women was ranged from 25-80 years old with a mean of 49.21 (SD+0.67) years. The majority of the age group was 45-55 years and represented 31.1%.

![Figure 1: Patients age distribution during 2016-2018 years and age ranged from (25-80) years.](image-url)

Generally according to data collected and summarized in table 1, increasing in frequency of
patients age. However, the range of 45-55 years was recorded as the highest incidence rate, about 31.1% (88) of total patients compared to other age ranges.

Table 1: frequency distribution of patients according to age

<table>
<thead>
<tr>
<th>Patient age (Year)</th>
<th>No. of patient</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-35</td>
<td>37</td>
<td>13.2</td>
</tr>
<tr>
<td>35-34</td>
<td>74</td>
<td>27.9</td>
</tr>
<tr>
<td>45-55</td>
<td>88</td>
<td>31.1</td>
</tr>
<tr>
<td>55-65 and above</td>
<td>83</td>
<td>29.6</td>
</tr>
</tbody>
</table>

In table-2, illustrate the socio-demographic characteristics and clinical history of 282 female patients diagnosed with breast cancer. The peak frequency occurred among the age group 25-80 years. Selected equal patient age above and under 49 years were 141 females. About 65.6% of them were determined as married and 44% were having less than four children. Also, 52.8% having a mass on their right side of the breast. Their history of lactation was recorded in 70.2%, on the other hand, 72.7% doesn’t take contraceptive pills and 90.4% doesn’t have a smoking habit.

Table 2: socio-demographic characteristics of female patients with breast cancer.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n.)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (49.21±0.67)</td>
<td>141</td>
<td>50</td>
</tr>
<tr>
<td>&lt;49</td>
<td>141</td>
<td>50</td>
</tr>
<tr>
<td>&gt;49</td>
<td>282</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>268</td>
<td>95</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>25</td>
<td>8.9</td>
</tr>
<tr>
<td>Married</td>
<td>185</td>
<td>65.6</td>
</tr>
<tr>
<td>Widow</td>
<td>41</td>
<td>14.5</td>
</tr>
<tr>
<td>Divorced</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>268</td>
<td>95</td>
</tr>
<tr>
<td>Missing data</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>

Next, the histological trend and grade of common pathological type between patients with breast cancer were represented in table- 3. The predominant tumor histological patterns were: invasive ductal carcinoma 207 (74.4%), metastatic carcinoma 20 (7.1%), and invasive lobular carcinoma 13 (4.6%). On the other hand, the histological tumor grade was available for 121 patients (42.9%). The common grade was II in 88 patients (31.2%), grade III 25 (8.9%), and grade I 8 (2.2%). Also, stage II a recorded in 31 patients (11%), stage IIb in 22 (7.8%) followed by stage III c in 17 (6%).

While tested biomarkers faced almost missing data during recording follow up. The main problem for patients following up, and missing data recorded to reach 94% of the tested cases (data not shown). Even several types of biomarkers were available but need more following up documentation, and more control.
Table 3: Histological features of patients with breast cancer.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history with BCA</td>
<td>57</td>
<td>20.2</td>
</tr>
<tr>
<td>Yes</td>
<td>218</td>
<td>77.7</td>
</tr>
<tr>
<td>No</td>
<td>4276</td>
<td>97.9</td>
</tr>
<tr>
<td>Total</td>
<td>2171</td>
<td>49.3</td>
</tr>
<tr>
<td>Missing data</td>
<td>6</td>
<td>2.1</td>
</tr>
<tr>
<td>Histological diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>207</td>
<td>73.4</td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>13</td>
<td>4.6</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>20</td>
<td>7.1</td>
</tr>
<tr>
<td>Total</td>
<td>254</td>
<td>90.1</td>
</tr>
<tr>
<td>Missing data</td>
<td>28</td>
<td>9.9</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>2.8</td>
</tr>
<tr>
<td>II</td>
<td>88</td>
<td>31.2</td>
</tr>
<tr>
<td>III</td>
<td>25</td>
<td>8.9</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>42.9</td>
</tr>
<tr>
<td>Missing data</td>
<td>161</td>
<td>57.1</td>
</tr>
<tr>
<td>BI-RADs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II, III</td>
<td>21</td>
<td>7.4</td>
</tr>
<tr>
<td>IV, V</td>
<td>81</td>
<td>28.7</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>36.2</td>
</tr>
<tr>
<td>Missing data</td>
<td>180</td>
<td>63.8</td>
</tr>
<tr>
<td>Staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage Ia</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>Stage Ib</td>
<td>3</td>
<td>1.1</td>
</tr>
<tr>
<td>Stage IIa</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Stage IIb</td>
<td>22</td>
<td>7.8</td>
</tr>
<tr>
<td>Stage IIIa</td>
<td>12</td>
<td>4.3</td>
</tr>
<tr>
<td>Stage IIIb</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td>Stage IIIc</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Stage IV</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>36.2</td>
</tr>
<tr>
<td>Missing data</td>
<td>180</td>
<td>63.8</td>
</tr>
</tbody>
</table>

In an attempt to classify patients according to median age into those who above 49 years old and those who lower than 49 years old and data presented in the table- 4. Significant statistical differences were noticed regarding using contraceptive pills, smoking habits, marital status, and the number of having children. Patients <49 years age utilize significantly more contraceptive compared to those above 49 years old (P<0.024), the smoking habit was noticed in women >49 years old significantly more than those <49 years old (P<0.026). While the older age >49 years was prefer breastfeeding significantly higher than <49 years old (P<0.08), the number of having children >4 was significantly in <49 years old (P<0.044). The frequency of unmarried (single) women <49 years old was a significantly higher number than above 49 years old.

Table 4: demographic characteristics of the patients under study according to age (>49 years versus <49 years):

<table>
<thead>
<tr>
<th>Patients characterization</th>
<th>Age &lt;49 year</th>
<th>Age &gt;49 year</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>65</td>
<td>65</td>
<td>1.0</td>
</tr>
<tr>
<td>Right</td>
<td>74</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Using contraceptive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>5</td>
<td>0.024</td>
</tr>
<tr>
<td>No</td>
<td>105</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31</td>
<td>26</td>
<td>0.459</td>
</tr>
<tr>
<td>No</td>
<td>106</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>14</td>
<td>0.026</td>
</tr>
<tr>
<td>No</td>
<td>132</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Lactation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94</td>
<td>104</td>
<td>0.08</td>
</tr>
<tr>
<td>No</td>
<td>39</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>No. of children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>70</td>
<td>54</td>
<td>0.044</td>
</tr>
<tr>
<td>&lt;4</td>
<td>54</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>14</td>
<td>11</td>
<td>0.00</td>
</tr>
<tr>
<td>Married</td>
<td>111</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Widow</td>
<td>4</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>
On the other hand, couldn’t notice any statistical differences in respect to the clinicopathological data including histological tissue type, stage group, and tissue grading as presented in table-5. But patients under 49 years old were in stage two (a, b, and c) recorded in 18, 17, and 11 patients respectively. And grade II recorded in 51 patients under 49 years old.

**Table 5: Clinical characteristics of the patients diagnosed with breast cancer under study according to age (>49 years versus <49 years)**

<table>
<thead>
<tr>
<th>Patients characterization</th>
<th>Age &lt;49 year</th>
<th>Age &gt;49 year</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ib</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>18</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>17</td>
<td>5</td>
<td>0.12</td>
</tr>
<tr>
<td>IIIa</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>IIIc</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>4</td>
<td>0.77</td>
</tr>
<tr>
<td>II</td>
<td>51</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

In table-6, the comparison between tumor grading and staging was recorded in 93 patients, significant differences (P<0.001) was shown between grading and staging; grade II/ stage IIa was higher in 28 patients. There were significant statistical differences as shown in table-7, in comparing tumor histological type and grading (P<0.002). Ductal carcinoma has a higher number of patients among other types and stage IIa and stage IIb was recorded in 21 patients for both.

**Table 6: Comparison between grading and staging**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage Ia</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Stage Ib</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stage IIa</td>
<td>1</td>
<td>28</td>
<td>1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Stage IIb</td>
<td>1</td>
<td>19</td>
<td>2</td>
<td>22</td>
<td>0.001</td>
</tr>
<tr>
<td>Stage IIIa</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Stage IIIb</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stage IIIc</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>69</td>
<td>20</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>
Table 7: comparison between histological types and grading

<table>
<thead>
<tr>
<th>Variable</th>
<th>Benign</th>
<th>Ductal carcinoma</th>
<th>Lobular carcinoma</th>
<th>Metastatic carcinoma</th>
<th>total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage Ia</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Stage Ib</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Stage IIa</td>
<td>3</td>
<td>21</td>
<td>6</td>
<td>0</td>
<td>30</td>
<td>0.002</td>
</tr>
<tr>
<td>Stage IIb</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>1</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Stage IIIa</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Stage IIIC</td>
<td>0</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>77</td>
<td>13</td>
<td>7</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Breast cancer with estrogen receptor ER +ve accounts for three quarter percent of western women while Arab women have a majority in ER -ve expression with 20%, 30%, and 9.3% in the UAE, Saudi women, and Lebanese women respectively compared to 9% in European women (9%) (10).

Women in low- income countries especially as presented in the review of Munzone E (2014) (11), the study was focusing on Africa region were women seek treatment in advanced stages or when the tumor spread to other organs and care has a relief aspect in these women; while in comparison to European countries very few women were diagnosed in late stages of cancer. In Kenya and Uganda for example in the study, all women were diagnosed at a late stage and assumed to be associated with high mortality rates (11).

The tests for breast cancer which are commercially available and currently used in the clinical practice were: the Oncotype DX test (Genomic Health, Redwood, CA, USA), the MammaPrint test (Netherlands Cancer Institute™ and Agendia™, Netherland), and the Prosima one (NanoString Technologies, Seattle, WA, USA) very expensive and not very widely in use in our country (Iraq especially in Baghdad). The Oncotype DX test represent the most widely used molecular test in the therapeutic decision-making and predictive for endocrine responsiveness in hormone receptor-positive breast cancers with 0–3 positive nodes, also it is recommended by both the National Comprehensive Cancer Network (NCCN) and the St. Gallen Consensus (7).

In comparison between Arabic women and the United States, the recording average age of newly diagnosed breast cancer was high and estimated at 60 years old and these are the result of increases in the age specifically in the status of ER+ /PR+ in post-menopausal US women (12). While in Northern Iraq the status of incidence at age is different, it was recorded to be less 50 years old in Sulaimaniyah for Kurdish people (13),
the authors suggested that the increase in the age rate of breast cancer in Iraq and other Middle-East countries was might be the increased establishment of new cancer centers for early detection.

In the present retrospective study, results were parallel to published records and gives an idea about disease diagnosis and incidence rates that would help to ensure accurate records and next to proper disease diagnosis and management in the future. For sure, more studies with more significant periods and risk factors were required to explain more accurate findings.

Breast cancer management programs require multi-disciplinary teams starting from surgery, radiation and chemotherapy and that will need more resources and related expertise to identify genes associated with breast cancer that would need more accurate procedures and expenses a lot (14).

In Iraq, a preliminary analysis study carried by Alwan 2016, 855 patients were recruited and the study findings were: 35% of the women were diagnosed at the age of 45-54 years old, their history of lactation and hormonal therapy was reported in 48 %, and 20.5% respectively. Only 18.5 % of the group with breast cancer family history. Depending on TNM classification, 9.8% stage I of the disease and 46% at stage II and IV. Infiltration ductal carcinoma was the most common pathology about 67% (the same of this study findings), followed by ductal carcinoma, lobular carcinoma, and malignant tumor 13.6%, 18.5 %, and 7 % respectively (15).

In a recent study carried by Alwan and her team work 2019, 1172 females have participated in their study from Baghdad city. They found that the main age was 51 years old and the patient’s groupage under 50 years demonstrated about 46.8%. Participants with breast cancer family history were recorded in 18.7%. Stages I-IV were recorded in 12%, 47.5%, 31.9% and 8.6% respectively. The frequency of age at diagnosis was significantly estimated higher among younger women under 50 years old. Alwan and her colleague give a conclusion that “breast cancer in Iraq represent an advanced disease at the time of diagnosis that justifies the necessity to promote public awareness educational campaigns to strengthen our national early detection programs” (16).

Limitations

There were several limitations while collecting data in this study: 1) most of the results of the biomarker were not included in patents file records, 2) also most of the FNA result not included, 3) the most importantly missing the follow-up of each patient that undergo surgery and 4) wither patients started chemotherapy or hormonal therapy cycles. These limitations might effects Iraqi cancer registration processes for breast cancer future information also affects planning for the new strategy of facilities, Mammography and MRI devices have the ability to detect small mass and in situ breast cancer that might be helpful in early detection programs.

Also, the unavailability of breast cancer mortality records was faced during data collection, it would be a predictor of effects caused by any disease or complications. Asian countries as mentioned in the study (17) don’t have proper records and data of incidence rate and mortality were collected from hospital-based registries and records.

**Source of Funding:** Self

**Conflict of Interest:** Nil

**Ethical Clearance:** taken for this study from the Iraqi Center for Cancer and Medical Genetics Research/ Mustansiriyyah University, and conducted according to the criteria set by the declaration of Helsinki

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Knowledge about COVID-19 among Citizens in Thi-Qar Governorate/Iraq: an Online Survey

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Abstract

Epidemics of infectious disease, such as coronavirus disease 2019 (COVID-19), necessitates knowledge assessment of pertinent populations as fast as possible because the findings are of great importance to the public health response. This study aims to (i) Assess the knowledge of Iraqi residents about COVID-19 and (ii) Identify the relationship between certain demographic features and the overall knowledge. This study was conducted in Thi-Qar governorate/Iraq, the researcher used an electronic survey questionnaire (using google form), the Uniform Resource Locator was distributed through social media platforms with brief introduction concerning the study. The tool of the study (questionnaire) involved of two parts: demographics (4 items) and knowledge questionnaire (3 sub-domains including 12 items). The total correct answers rate was (88.1%). Lowest answers rate scored in routes of transmission sub-domain (82.5%) while control and prevention (92.7%) sub-domain scored the highest rate. Mean knowledge score of 10.57 (standard deviation: 1.5, Range: 0-12). Also, results display highly significant relationships between overall knowledge and demographic characteristics (P <0.001).

Conclusion: Most well-educated Iraqi residents, particularly males, are knowledgeable about COVID-19. However, this result should be generalized with caution due to limited sample representativeness.

Keywords: COVID-19, Coronavirus disease, Knowledge, Control, Iraq.

Introduction

COVID-19 was reported for the first time in Wuhan, China, in December 2019 as unknown cause pneumonia cases in a cluster of individuals (1). On 11th of March 2020, the World Health Organization (WHO) declared Covid-19 a pandemic (2) and by June 30th 2020, there were 10,185,374 confirmed cases and 503,862 reported deaths internationally (3). Therefore, an organized universal reaction is needed urgently to help in preparation of health systems to encounter this exceptional challenge. Even though the measures of containment applied in China have lowered new incidents by over than 90%, the situation is different in other countries (ex: Italy and Iran) (4)

Studies identified various risky groups, infection appears to be more severe in older adults, obese people, and individuals with previous medical conditions. However, severity of infections during pregnancy have not been reported, and a small number of incidents have been reported in children (5). Majority of COVID-19 patients have exhibited mild manifestations including sore throat, fever, and dry cough. Most of cases have cured spontaneously. Still, some have showed multiple deadly complications such as septic shock, pulmonary edema, severe pneumonia, organ failure, and Acute Respiratory Distress Syndrome (ARDS) (6)

Multiple actions can be taken to contain an emergent infection such as COVID-19. Globally, the previous months has seen an increasing countries number that declare precautions on travel or complete entry prohibitions on individuals from certain affected areas. These restrictions of mobility can be evaluated to confirm their potential efficiency in hindering local
epidemics (7). Furthermore, it may give an idea how and when to stop such restrictions (8).

In Iraq, various exceptional actions have been taken in order to delay the spread of COVID-19, including schools and universities closure, mass transportation prohibition, public spaces lock up, prevention of Human gatherings, and separation and care for people with infection and suspected individuals. In March 17, government had declared complete curfew, and started Awareness campaigns for people to stay at home and avoid unnecessary contact with each other.

Covid-19 pandemic course is highly impacted by the way individuals behave, and that is affected by what their knowledge and beliefs about this disease (9). Misinformed people in regard to COVID-19 spread (especially through social media) needs an urgent and serious handling, and that’s why WHO has established “myth busters” page on their website (10).

The world still continuing to fight against COVID-19 to ensure the ultimate victory, and the commitment and adherence of population to the measures of control are indispensable. previous experience with the 2003 SARS outbreak recommend that fright sensation among the individuals from infectious diseases will have negative effects toward knowledge and attitudes, and this have further confuse efforts to control the infestation of the disease (11,12). This needs quick population’s assessments of knowledge about COVID-19 (13). In this study, authors aimed to investigate the knowledge about COVID-19 of Iraqi citizens in Thi-Qar governorate and to Identify any significant relationship between certain demographic characteristics with the overall knowledge.

Methods

Design, sitting, and sample

Cross-sectional study was conducted from 10 to 22 of April in Thi-Qar governorate, and because of this special circumstances, population-based assessment was not feasible. So, authors collected the data online using random sampling technique. Goggle form was prepared and the Uniform Resource Locator (URL) was distributed through social media platforms (i.e. Facebook, WhatsApp, WeChat, Twitter) counting on the authors’ connections with local citizens living in Thi-Qar governorate. Also, recruitment poster was posted on official accounts of Thi-Qar health directorate on previously mentioned platforms, with brief information about importance, aims, measures, voluntary contribution with anonymity, and notes about how to fill the questionnaire. Contributors have to choose yes or no options to approve their readiness for voluntary participation. Individuals of Iraqi nationality who aged 15 years or more were directed to answer the questionnaire.

Instruments

The tool of the study (questionnaire) involved of two parts: demographics and knowledge questionnaire. Demographic characteristics involves (gender, age, educational level, and source of information about COVID-19), knowledge questionnaire was adapted from (14) with permission. The authors translated the questionnaire into Arabic following the recommended guidelines (15), internal consistency was at acceptable level (the alpha-Cronbach coefficient of the questionnaire was 0.83).

Knowledge questionnaire is consisted of 12 items: clinical manifestations (4 items) (ex: Fever, dry cough, myalgia, and fatigue are the chief clinical manifestations of COVID-19 infection), routes of transmission (3 items) (ex: Eating or contacting wild animals would cause COVID-19 infection), and control and prevention (5 items) (Isolation and treatment of people who are infected with the COVID-19 virus are effective ways to reduce the spread of the virus). True, false, and I don’t know options were provided as answers. 1 point awarded to correct answer and for incorrect/I don’t know answers, 0 point was awarded. Higher score indicates higher knowledge.

Statistical Analysis

The analysis of data was accomplished through the usage of statistical package for social sciences (SPSS) version 26. Frequency, percentage, mean, and standard deviation were used. Independent sample t-test and Analysis of variance (ANOVA) were used to determine differences in mean knowledge scores among groups as convenient, Multiple linear regression was conducted to identify demographic factors association with overall knowledge.
Results

Demographic Characteristics

Study involved the contribution of 5280 participants. Majority of study sample were males (65.8%) with (55%) of 15-29 age group. (41.9%) of participant have bachelor degree, and (45.6%) were depending on social media as a source for information about COVID-19. Other demographics are shown in (Table 1).

Knowledge Score

The correct answers rate was (88.1%), sub-domains show different rates as follow: clinical manifestations (86.6%), routes of transmission (82.5%), and control and prevention (92.7%). Item 10 was the highest correct answered item (98.3%) while item 6 was the lowest with (74.1%), other individual rates of correct answers as shown in Table 2. The mean knowledge score about COVID-19 was 10.57 (standard deviation: 1.5, Range: 0-12).

Table (3) Predictors of significance to participant knowledge in this study were age, gender, educational level, and source of information about COVID-19. Age was the factor of highest effect on overall knowledge ($\beta = .234; p <.001$), followed by gender ($\beta = -.202; p <.001$), educational level ($\beta = .131; p <.001$), and source of information about COVID ($\beta = .105; p <.001$).

Table (1) knowledge score by socio-demographic variables of participants (N = 5280)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of participants (%)</th>
<th>Knowledge score M (SD)</th>
<th>t/F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29 years</td>
<td>2904 (55)</td>
<td>11.07 (1.58)</td>
<td>7.513</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30-49 years</td>
<td>2211 (41.9)</td>
<td>10.44 (1.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 years and above</td>
<td>165 (3.1)</td>
<td>10.19 (1.69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3475 (65.8)</td>
<td>10.11 (1.92)</td>
<td>4.787</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>1805 (34.2)</td>
<td>11.02 (1.54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary school and below</td>
<td>936 (17.7)</td>
<td>9.48 (1.19)</td>
<td>20.821</td>
<td>0.013</td>
</tr>
<tr>
<td>Associate degree</td>
<td>1132 (21.4)</td>
<td>10.28 (1.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bachelor degree</td>
<td>2210 (41.9)</td>
<td>11.12 (0.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Master degree and above</td>
<td>1002 (19)</td>
<td>11.37 (1.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Source of information about COVID-19</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social media</td>
<td>2409 (45.6)</td>
<td>9.56 (1.71)</td>
<td>9.525</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Official governmental and health sources</td>
<td>2211 (41.9)</td>
<td>11.62 (1.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television</td>
<td>627 (11.9)</td>
<td>11.38 (1.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family and friends</td>
<td>33 (0.6)</td>
<td>9.72 (1.58)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (2) COVID-19 knowledge questionnaire

<table>
<thead>
<tr>
<th>Items</th>
<th>Percentage of correct answers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fever, dry cough, myalgia, and fatigue are the chief clinical manifestations of COVID-19 infection</td>
<td>93.8</td>
</tr>
<tr>
<td>2. Unlike the common cold, runny nose, stuffy nose, and sneezing are less common in individuals infected with the COVID-19 virus.</td>
<td>83.3</td>
</tr>
<tr>
<td>3. There is no effective cure for COVID-19 currently, but initial supportive and symptomatic treatment can assist majority of patients to recover from the infection</td>
<td>89.4</td>
</tr>
<tr>
<td>4. Not all cases of COVID-19 will progress to severe cases. usually old age people, who have chronic diseases, and who are obese are more expected to develop severe cases</td>
<td>79.8</td>
</tr>
<tr>
<td>5. Eating or contacting wild animals would cause COVID-19 infection</td>
<td>78.2</td>
</tr>
<tr>
<td>6. When fever disappears, individuals with COVID-19 cannot transmit the virus to others</td>
<td>74.1</td>
</tr>
<tr>
<td>7. COVID-19 virus spreads through respiratory droplets of infected individuals.</td>
<td>95.2</td>
</tr>
<tr>
<td>8. Residents can use general medical masks to avoid infection with COVID-19</td>
<td>82.5</td>
</tr>
<tr>
<td>9. children and young adults are not required to take actions to avoid infection by COVID-19 virus</td>
<td>90.4</td>
</tr>
<tr>
<td>10. Individuals should avoid going to overcrowded places such as train stations and stop taking public transportations to prevent the infection by COVID-19</td>
<td>98.3</td>
</tr>
<tr>
<td>11. Isolation and treatment of people who are infected with the COVID-19 virus are effective ways to reduce the spread of the virus</td>
<td>95.4</td>
</tr>
<tr>
<td>12. People who have contact with someone infected with the COVID-19 virus should be immediately isolated in a proper place. In general, the observation period is 14 days</td>
<td>97.1</td>
</tr>
</tbody>
</table>

Table (3) Relationship between total knowledge and demographic characteristic

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>.779</td>
<td>.008</td>
<td></td>
<td>93.950</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>-.053</td>
<td>.003</td>
<td>-.202</td>
<td>-15.594</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>.055</td>
<td>.004</td>
<td>.234</td>
<td>14.437</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Educational level</td>
<td>.036</td>
<td>.003</td>
<td>.131</td>
<td>5.946</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Source of information about COVID-19</td>
<td>.020</td>
<td>.002</td>
<td>.105</td>
<td>8.091</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

B= unstandardized coefficients, SE= Standard error of B, β= standardized coefficients, t: t-test.
Discussion

Rapid and unpredictable spread of COVID-19 disease made it the most international pressing health issue currently with a close follow up from the concerned authorities. Also, this disease has become a cross topic for the public with a lot of inaccurate material from variety of sources, and that reflect the importance of taking the reliable information and apply it in our daily actions. Hence, good base of knowledge will help in the eradication efforts and in spreading of healthy behavior among the population.

As far as the author know, this is the first study to assess the knowledge of residents about COVID-19 in Iraq. The findings exhibit average correct rate of 88.1% in the questionnaire compared to studies from America and China which show approximate rates of 80% and 90% respectively (14,16). Our results reflect that majority of sample have a good level of knowledge. This may be related to the fact that majority of sample was well-educated and have the proper awareness to look for accurate information from its official sources and this assumption is supported by the significant positive association between knowledge scores on COVID-19 and educational level. Also, this study was conducted after approximately 1 month of pandemic declaration by WHO and a significant amount of information were published to the public during this period.

From the beginning of this crises, Iraqi authorities started awareness campaigns through all media platforms to ensure that individuals commit to international guidelines on COVID-19. However, not all of the community is expected to percept these instructions equally and some of the underprivileged groups such as emigrants, poor people, and residents of rural areas may even not have the means to reach for such information. Therefore, knowledge about COVID-19 in such vulnerable groups needs superior attention by both authorities and researchers. Factors such as poor socioeconomic status and low educational levels may be a determinants of knowledge levels (17). Specific educational programs can be tailored to target specific populations and may be of a huge benefit in increasing knowledge levels.

Individuals who depend on social media and on family and friends as a source of information have the lower knowledge score (9.04 and 9.10 respectively) in comparison to those who takes information from official sources (11.10) and television (10.96). This result emphasize on the importance to investigate the accuracy of information on COVID-19, responsible health authorities and relevant studies advised that widespread unsupported information about COVID-19 is a significant issue that may cause xenophobia internationally (18,19).

Other findings show significant association between demographic variables and overall level of knowledge. Age was the factor of highest effect on overall knowledge, followed by gender, educational level, and source of information about COVID. This result is reinforced by multiple studies that have disclosed that older, females, and higher educated respondents are more knowledgeable about emerging communicable diseases (14,20,21,22). However, even with the large sample in this study which may be considered as a favorable point, sample was more demonstrative of male and well-educated participants. So, the results may be generalized with caution.

Multiple limitation was encountered during this study, one of them is that due to limited internet access and low socioeconomic status, some groups were less representative in the study sample as mentioned above. Also, study was limited to one governorate in Iraq and that is another reason why findings are not fully representative. Another one concerning that the data presented in this study depends on participants recall of information which may be subjected to bias.

Conclusion/Recommendation

Most well-educated Iraqi residents, particularly males, are knowledgeable about COVID-19. However, this result should be generalized with caution due to limited sample representativeness. Authors recommend that wider and more representative sample should be studied to get more applicable results.

Acknowledgements: The author would like to thank all the participant of the study for their treasured time. Also, great thanks to Dr. Yi Li for his valuable assistant during questionnaire preparation.

Conflicting Interests: No conflicts of interest declared by the authors.

Funding: The author disclosed receipt of no funding.
Ethical Permissions

Ethical Board of Thi-Qar health directorate permitted the study plan and procedures [Reference no: 33/3588 in 23/4/2020]. Informed consent was introduced before the official questionnaire were offered. Contributors have to choose yes or no options to approve their readiness for voluntary participation.

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Study of Prevalence of Placenta Previa and Circumstances among Pregnant Women in Fallujah Hospital

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¹PhD. M.D. Lecturer, ²Lecturer, ³M.D. Lecturer, College of Medicine, University of Fallujah, ⁴M.D. Resident AL- Fallujah Teaching Hospital, AL Fallujah, Iraq

Abstract

Placenta previa refers to placenta which is totally or partly implanted in lower segment of the uterus. Mortality and morbidity of mother increase with placenta previa due to the increase in the incidence of bleeding during pregnancy. This study aims to define placenta previa prevalence among pregnant women at AL-Fallujah teaching hospital and to determine the risk factors and their association with placenta previa incidence in al-Fallujah city population.

This retrospective cohort study investigated medical registry of 6339 gravid women in al-Fallujah teaching Hospital in Iraq. We find 13 cases of placenta previa (0.21%) amongst the 6339 cases registered. The risk factors that strongly appeared to affect the complication of pregnancy with placenta previa were; advanced maternal age of ≥ 35, parity, previous curettage and previous cesarean section (P<0.01).

The most important pregnancy outcomes of the placenta previa were lost blood of mother more than five hundred cubic centimeter (P = 0.000) and Apgar score of the baby at first minute equal or less than 7 (P = 0.003), Placenta accreta and cesarean hysterectomy (P< 0.01).

These findings are the same as those recorded in other research for Asian, American and European gravid women, with some other detected factors.

Keywords: Prevalence, Placenta previa, Risk factors, maternal age, Parity, Previous curettage, previous cesarean section

Introduction

Placenta previa refers to placenta which is totally or partly implanted in lower segment of the uterus. Around 1/3 of the ante partum bleeding is due to placenta previa. The classical features of placenta previa are usually bleeding which could be abrupt starting, recurrent, no pain, apparently no cause ¹. Mortality and morbidity of mother will increase with placenta previa, this is due to raise in the incidence of bleeding during pregnancy, hemorrhagic shock with its sequel as circulatory hypovolemia , anemia, with long staying in hospital. Placenta previa also can be a cause of increasing in the incidence of operative interference, postpartum bleeding and sepsis ¹.

Other risks associated with placenta previa are: around four fold increases in the risk of 2nd trimester vaginal hemorrhage, peripartum hysterectomy, transfusion of blood, and accreta placentaæ ².

Placenta previa can be a cause of preterm delivery which is associated with higher incidence of perinatal morbidity and mortality. Preterm delivery is associated with many complications as neonatal sepsis, asphyxia at birth and birth weight less than 10th centile for gestational age. Placenta previa incidence is about 1 in 300 deliveries ¹,³. Maternal age is related to the risk of placenta previa , women who are 19 years old or less its incidence around 1 in 1500 and for women aged 35 years or more it is 1 in 100 ⁴. Multiparity, previous cesarean scar increase the possibility of placenta previa ⁵. Incidence
accreta is a remarkable risk factor for the occurrence of placenta previa. A previous cesarean operation is considered the most significant factor for placenta previa, including presentation, hemorrhage, and morbidity of the mother. The uterine serosa and more invasion, more risks for percreta refer to invasion of myometrium reaching the endometrium, and embolization of uterine artery. Increta refer to deep invasion of myometrium while superficial invasion of myometrium by placental villi leads to a cesarean hysterectomy as the leading reason for postpartum bleeding and indication for a cesarean hysterectomy. Usually, accreta refer to superficial invasion of myometrium by placental villi, increta refer to deep invasion of myometrium while percreta refer to invasion of myometrium reaching the uterine serosa and more invasion, more risks for hemorrhage and morbidity of the mother. Predisposing factors for placenta accreta could be uterine curettage, surgery by hysteroscope, myomectomy a trial of endometrium, and embolization of uterine artery. A previous cesarean operation is considered the most remarkable risk factor for the occurrence of placenta accreta and the continual increase in the cesarean operation rates throughout the world insures that accretas will stay a bothersome clinical matter. The risk for accreta occurrence is significantly increased if the woman has a history of a prior Cesarean operation and the placenta previa at same time.

**Methods**

Ethical approval was taken from AL Fallujah teaching hospital, a retrospective rehearsal was done for in-patient medical register of gravid women with singleton pregnancy (running state respectively) who were gestational age 28 weeks and delivered at AL-Falluja Hospital was studied. Last menstrual period (LMP) was used for estimation of the gestational age or dating US (ultrasound) for women with unknown or uncertain LMP. The cases which were excluded include multiple pregnancies, uncertain gestational age which is not approved by US dating and women with incomplete medical record. Location of placenta was determined before the delivery by US during the 3rd trimester at 28 weeks’ gestation or during the operation, visualization of placenta previa during cesarean delivery, including 2 types of placenta previa which are placenta covers the internal osseous partially or completely (previously, these were classified as total centralis or partial centralis) and low-lying placenta which refer to implantation in the lower uterine segment (placental edge does not cover the internal osseous but lies within a two centimeters wide perimeter around the osseous). We reviewed prenatal record of all pregnant women in our sample population, their labor and delivery visits in order to extract the maternal medical and obstetric complications and demographic features, as well as factors related to placenta previa. The variables which are evaluated including age of the mother at time of delivery, parity, prior cesarean operation, uterine curettage, obstetric complications, underlying disease, weeks of pregnancy at birth, Apgar score and gender of newborn. We performed Statistical analysis using the IBM SPSS statistics 26.Inc for Windows. At first a descriptive statistics for each variable was done which included frequencies for categorical variables and average value with standard deviation for continuous variables. Chi-Square Test of Independence (also called Chi-Square Test of Association) which determines whether there is an association between categorical variables (i.e., whether the variables are independent or related) was used to compare cases with and without placenta previa. P value of 0.01 or less was considered highly statistically significant in this study.

**Results**

Data used for this study was medical records of
6339 pregnant women collected between October 2018 and March 2019 from AL-Fallujah teaching hospital. It was found that placenta previa affected 13 (0.21%) of the 6339 pregnancy cases analyzed in this study. It was noticed that the placenta previa prevalence is higher among women whom age was more than 35 years or were multiparous, or had a previous cesarean labor, or had previous obstetrical complications. Table 1 shows the main characteristics of the study population. At delivery the mean maternal age was 26.5 ± 6.65 years old, while nearly half of the study population was nulliparous (52.6%), on the other hand the percentage of delivery after 37 weeks of gestation was the predominant and comprises (88.1%). For the methods of labor, 62.7% were normal vertex labor, 24.5% for emergency cesarean operation, 10.1% for elective cesarean section and 2.7% for vaginal breech assisting mode. The baby mean weight at birth was 3,049.9 ± 496.1 grams. Table 2 indicates the factors that are related to placenta previa. Major differences were noted among pregnancies with and without placenta previa for the following factors maternal age, parity, previous curettage and previous cesarean operation where these factors showed strong association (P value <0.01) with placenta previa.

Table 3 displays the frequency of different maternal complications and pregnancy outcomes in women with and without placenta previa. Generally, it was noticed that complications included placenta accreta, cesarean hysterectomy, 1-minute and 5-minute Apgar score less than 7, and neonatal intensive care unit admission, are more possible to happen to cases with placenta previa. Also it can be observed from table 3 that lost blood of mother more than five hundred cubic centimeter (P = 0.000), placenta accreta (P value = 0.000), previous cesarean hysterectomy (P value = 0.000) and Apgar score at 1st minute (P value = 0.003) were strongly related to placenta previa.

<table>
<thead>
<tr>
<th>Table 1. Basic characteristics of 6339 pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Age(year)</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
</tr>
<tr>
<td>pre-term &lt;37</td>
</tr>
<tr>
<td>term ≥37</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>≥1</td>
</tr>
<tr>
<td>Spontaneous vertex labour</td>
</tr>
<tr>
<td>Mode of delivery</td>
</tr>
<tr>
<td>Emergency cesarean operation</td>
</tr>
<tr>
<td>Elective cesarean operation</td>
</tr>
<tr>
<td>Vaginal breech assisting</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Baby birth weight (g)†</td>
</tr>
</tbody>
</table>

†= low birth weight less than 2500 gram
### Table 2. Factors associated with placenta previa

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placenta previa N (%)</th>
<th>Non-placenta previa N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>(6) 46%</td>
<td>(5272) 83.3%</td>
<td>0.000*</td>
</tr>
<tr>
<td>≥35</td>
<td>(7) 54%</td>
<td>(1054) 16.7%</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>(2) 15.4%</td>
<td>(3363) 53.2%</td>
<td>0.006*</td>
</tr>
<tr>
<td>%</td>
<td>(11) 84.6%</td>
<td>(2963) 46.8%</td>
<td></td>
</tr>
<tr>
<td>Multipara</td>
<td>(6) 54%</td>
<td>(5625) 88.92%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(46)</td>
<td>(701) 11.08%</td>
<td></td>
</tr>
<tr>
<td>Previous curettage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(7) 54%</td>
<td>(5625) 88.92%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(46)</td>
<td>(701) 11.08%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(6) 46%</td>
<td>(5625) 88.92%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(46)</td>
<td>(701) 11.08%</td>
<td></td>
</tr>
<tr>
<td>Previous cesarean section</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(4) 30.8%</td>
<td>(4429) 70%</td>
<td>0.002*</td>
</tr>
<tr>
<td>%</td>
<td>(9) 69.2%</td>
<td>(1897) 30%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(11) 54%</td>
<td>(5024) 46%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(11) 54%</td>
<td>(5024) 46%</td>
<td></td>
</tr>
<tr>
<td>Medical complications †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(10) 76.9%</td>
<td>(5946) 94%</td>
<td>0.010</td>
</tr>
<tr>
<td>%</td>
<td>(3) 23.1%</td>
<td>(380) 6%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(6) 54%</td>
<td>(5625) 88.92%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(46)</td>
<td>(701) 11.08%</td>
<td></td>
</tr>
<tr>
<td>Obstetric complication ‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(5) 38.5%</td>
<td>(3762) 59.5%</td>
<td>0.123</td>
</tr>
<tr>
<td>%</td>
<td>(8) 61.5%</td>
<td>(2564) 40.5%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(6) 46%</td>
<td>(5625) 88.92%</td>
<td>0.703</td>
</tr>
<tr>
<td>%</td>
<td>(46)</td>
<td>(701) 11.08%</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(13) 100%</td>
<td>(6256) 98.9%</td>
<td>0.703</td>
</tr>
<tr>
<td>%</td>
<td>(0) 0%</td>
<td>(70) 1.1%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(2) 54%</td>
<td>(361) 5.4%</td>
<td>0.703</td>
</tr>
<tr>
<td>%</td>
<td>(7) 46%</td>
<td>(361) 5.4%</td>
<td></td>
</tr>
</tbody>
</table>

† = pregnant women who had underlying of medical disease such as; thalasemia, thyroid disorder, respiratory disease, heart disease, autoimmune disease and other diseases

‡ = pregnant women who present with obstetric complication such as; gestational diabetes, pregnancy induced hypertension

### Table 3. Pregnancy outcomes of study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placenta previa N (%)</th>
<th>Non-placenta previa N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37</td>
<td>(1) 7.7%</td>
<td>(632) 9.99%</td>
<td>0.782</td>
</tr>
<tr>
<td>≥37</td>
<td>(12) 92.3%</td>
<td>(5694) 90.01%</td>
<td></td>
</tr>
<tr>
<td>Gender of baby</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>(6) 46%</td>
<td>(3153) 49.8%</td>
<td>0.790</td>
</tr>
<tr>
<td>male</td>
<td>(7) 54%</td>
<td>(3173) 50.2%</td>
<td></td>
</tr>
<tr>
<td>Placenta accreta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(6) 46%</td>
<td>(6324) 99.97%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(7) 54%</td>
<td>(2) 0.03%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(7) 54%</td>
<td>(6324) 99.97%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(54)</td>
<td>(2) 0.03%</td>
<td></td>
</tr>
<tr>
<td>Cesarean hysterectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>(8) 61.5%</td>
<td>(6322) 99.94%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(5) 38.5%</td>
<td>(4) 0.06%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(6) 46%</td>
<td>(5693) 90%</td>
<td>0.000*</td>
</tr>
<tr>
<td>%</td>
<td>(7) 54%</td>
<td>(633) 10%</td>
<td></td>
</tr>
</tbody>
</table>

≤ 500  
> 500
Apgar score at first minute

| ≤ 7      | (3) 23.1% | (316) 5%  | 0.003* |
|>7        | (10) 76.9%|(6010) 95% |

Apgar score at fifth minute

| ≤ 7      | (1) 7.7%  | (63) 1%   | 0.016  |
|>7        | (12) 92.3%|(6263) 99% |

Small for gestational age

| No       | (11) 84.6%| (5630) 89%| 0.614  |
| Yes      | (2) 15.4% | (696) 21% |

NICU admission

| No       | (12) 92.3%| (6206) 98.1%| 0.102 |
| Yes      | (1) 7.7%  | (120) 1.9%  |       |

NICU = neonatal intensive care unit, mL= milliliter
* = significant at 0.01 level

### Discussion

Currently, one of the most popular causes of antepartum hemorrhage which might lead to maternal death is placenta previa. This is the reason why it should be diagnosed earlier in order to improve maternal outcome. This condition is usually diagnosed either by using transvaginal ultrasonography or transabdominal sonography. Some of the most associated factors to this condition are previous cesarean delivery, multiparity, previous curettage, and maternal age. The results of this study showed similarity to the prevalence of placenta previa in the population of Asia and in the same span of previous research. It was found that previous history of previous curettage, previous cesarean operation, multiparity and maternal ages were strongly associated with placenta previa. The effect of maternal age on the prevalence of placenta previa is probably due to the aging of uterus and the influence of recurrent pregnancies. This inference is of clinical importance for women who are delaying childbearing and decide to have children at a later or future time. On the other hand, unlike previous research, common risk factors like smoking was proved to be not related with placenta previa in our study. The reason behind this is that it is uncommon for Iraqi women to smoke as it is obvious from data only 70 of 6339 pregnant women had been reported having experience of smoking.

The results showed that the relation between parity and the incidence of placenta previa is significant; the explanation of this might be because Fallujah families are having more than 3 children.

Although our results showed that preterm delivery before 37 weeks’ and small birth weight are not related to placenta previa, it is known that placenta previa can be a cause of preterm labor and delivery which is considered an important reason of perinatal morbidity and death particularly lung immaturity and asphyxia at birth. This might be explained by the early diagnosis of placenta previa which might lead to permanence of pregnancy to term with no complication. Nevertheless, it is hard to confirm that the association of these factors same as previous research.

There were some limitations identified in this study. First, the percentage of placenta previa may have been underestimated because of the inaccurate birth certificates and data of hospital discharge, thus since our study is hospital-based it may not represent the prevalence of placenta previa in al- Fallujah community. The second limitation is that this study might lack for some information, for example, factors like total weight gain, uterine fibroids the use of reproductive technology, working throughout pregnancy, and body mass index.

- Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1
Conclusion

In this study the effect of risk factors on the frequency of placenta previa was examined. It was found that the prevalence of placenta previa was 0.21% among Fallujah city pregnant women population. Previous uterine curettage, previous cesarean section, maternal age and multiparity showed to be strongly associated with placenta previa.

Ethical Clearance: taken from the Scientific Committee in College of Medicine University of Fallujah.

Source of Funding: Self

Conflict of Interest: Nil

References

Assessment the effects of *Mastic Gum Resin*, *Lawsonia Inermis* and *Quercus Brantii* on Cutaneous Wound Healing in *BALB/c* Mice

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Abstract

The request for more efficient and lower-cost therapeutic methods for wound healing remains a challenge for modern medicine. The goals of this investigation were to recognize and look at the impacts of *Mastic Gum Resin, Lawsonia Inermis*, and *Quercus Brantii* in wound healing by using histopathological study and blood parameters in *BALB/c* Mice. Mice were comprised into four groups: Control negative group (n=10), mice were not treated with plant suspension only applied with normal saline; Treatment group I, mice were applied by *Mastic Gum Resin (MGR)* (n=10), Treatment group II, mice were applied with the *Quercus Brantii* (n=10), and the last group, Treatment III which were applied with *Lawsonia Inermis* (LI) (n=10). One ml for each suspension of *Mastic Gum Resin (MGR)*, *Quercus Brantii (QB)*, and *Lawsonia Inermis (LI)* was applied to the wound directly without suturing for 4 days/week for about 3 weeks. Wound healing effects were evaluated by utilizing the hematological profile for each group with the histopathological study. The cutaneous wound in *Mastic Gum Resin* and *Quercus Brantii* treated groups were more effective in progressing wound healing than *Lawsonia Inermis* treated group regarding histological changes at day 8 and day 18, respectively and blood parameters at day 21.

Keywords: Albino mice, Lawsonia Inermis, Mastic Gum Resin, Quercus Brantii.

Introduction

Wound healing is an unpredictable procedure that requires a progression of biochemical and cell responses, beginning with homeostasis, re-epithelialization, granulation tissue arrangement, and renovating of the extracellular matrix ¹,². Searches for better and reliable wound healing agents from medicinal plants have become more relevant fields of active research. From time immemorial, wounds were treated topically with various medicinal herbs or their extracts, according to conventional medicine ³. The oleoresin of *Pistacia atlantica* or “ mastic tar” is normally utilized in Pakistan and Iran to treat conditions, for example, gastrointestinal issues ⁴. This natural plant has caught the consideration of investigators because of studies on various areas of this plant, for example, the leaves, bits, frames, and gum showing different organic advantages, for example, antioxidant, antimicrobial, and anti-inflammatory effect. It has been demonstrated that *Pistachio* species are a rich source of phenolic mixes and they have additionally been discovered a source of durable antioxidant ⁵. *Mastic oleoresin* (MO) is fundamentally directed topically (as wound dressing), orally, or by smoking ⁶. Much examination has indicated the constructive outcomes of MO on wound curative ⁷. Other than that, MO has been applied straightforwardly to skin incisions due to its adhesive consistency ⁸. The oaks (genus Quercus) are among the most major groups of flowering plants that dominate large areas of the northern hemisphere. There are even more than 200 species of oak in the western

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hemisphere, and a potentially greater number in Asia and fairly few in Europe. The principal significance of the oak tree is the existence of tannins in its various parts. Due to the different characteristics of the tannins present in them, Quercus species can be used in wound treatment according to references and local knowledge. The gall extracts of this plant were historically used on inflamed skin and skin burn in Kurdistan but there are little details about the underlying mechanism. Phenolic compounds, tannic acid, fatty acid, and gallic acid are the major components of The Lawsonia inermis (henna). This plant considered to have certain restorative properties, for example, antibacterial, antioxidant, and anti-inflammatory effects, oral and effective use of Lawsonia Inermis (LI) leaves and its isolated part exhibited wound curative conduct in excision and incision wound models. The aims of this examination were to distinguish and analyze the impacts of Mastic Gum Resin, Lawsonia Inermis, and Quercus Brantii in wound healing in BALB/c mice by utilizing blood parameters and histopathological study.

Materials and Methods

Collection and preparation of plant material

Fresh Mastic Gum Resin, Lawsonia Inermis, and Quercus Brantii were collected from a local market in Sulaimnai city of Kurdistan provinces, Iraq. Two gm of Mastic gum Resin was applied directly without mixing with any materials to the wound area, while the Lawsonia Inermis, and Quercus Brantii (grinded by a mixer till become a powder) were prepared by adding 2 gm of each of them with 4ml of normal saline and mixed by stirrer till become the creamy or suspension like. Each mouse was received 200mg/kg.bw from each types of plant.

Animals

Forty BALB/c mice (six weeks age, male and female with bodyweight 20-40 grams) were housed in the veterinary teaching hospital animal lab, Sulaimani province. Animals were having free access for food and drinking water and they were maintained during the research time on a 12 hours light and dark cycle. The exploration was done with the authorization of the Ethics Committee (1237) at the College of Veterinary Medicine, University of Sulaimani, under the counsel and rules of the College of Veterinary Medicine for the Maintenance and Use of Laboratory Animals.

Experimental design

All surgical procedure was performed under Isoflurane general anesthesia, and all attempts were made to reduce suffering. The surgical procedure was performed in BALB/C mice in an operating room with very good facilities. After 1 week of acclimation, the back of the animal was shaved and a full-thickness skin was incised (1cm x 1cm) from the dorsum as seen in figure 1.

Mice were allotted into four groups: Control negative group (n=10), mice were not treated with plant suspension only applied with normal saline; Treatment group I, mice was applied by Lawsonia Inermis (LI) (n=10), Treatment group II, mice were applied with the Mastic Gum Resin (MGR) (n=10), and the last group, Treatment III which were applied with Quercus Brantii (n=10). Each suspension 200mg/ml was applied to the wound area by sticks directly without suturing for 4 days/week for about 3 weeks. No dressing was used.

Figure 1: The back incised wound.
Histological procedure

The skin biopsies were taken from each wound side on day 8 and 21. Tissue biopsies were fixed in 10% neutral formalin buffer for 48 hours and afterward prepared by paraffin-embedding procedure. Tissue sections 4μm thick were made utilizing a microtome (Leica, Germany). One slide from each example at every period was recolored with hematoxylin and eosin for distinguishing any histological features of the injured tissue. The tissue areas were analyzed by light microscopy and photographed utilizing a camera (Amscope™, Japan).

Haematological Study:

On day 21, animals were sacrificed under general anesthesia and peripheral blood was collected from the tail vein with Ethylene Di-amine Tetra Acetic acid (EDTA). The vacuumed blood collection tubes were shaken immediately to mix well and were analyzed directly. The total white blood cells (WBC) and differential white blood cells [lymphocytes (LYM), granulocytes (GRA)] and Minimum Inhibitory Dilution, a measure of rare cells and several precursor white cells (MID), total red blood cells (RBC), hemoglobin (HGB), packed cell volume (PCV), and platelets in each sample were measured by automatic hematology analyzer (Medonic M-series M32, Sweden).

Results

All mice survived and even no complications reported, including infection, correlated to the method was detected.

1. Histopathological Study:
   a. Microscopical assessments of cutaneous wound healing on days eight:

   On day 8th after wounding, the histological assessment of healed wound area shown inflammatory phase, on the surface, necrosis of skin tissue was placed as a consequence of mechanical damage, a continuous layer of marked granulation (Inflammatory cell, collagen deposition, and angiogenesis) tissue across the entire wound gap and depth, in which the thickness of the granulation tissues was higher in surface layer than in dermal layer, moreover, collagen fibers arranged in a disorganized manner and randomly distributed as fibrils with intense angiogenesis which were a main features of the granulation tissue, the intense inflammatory reaction (inflammatory cells including, neutrophils, macrophages, lymphocytes, plasma cells, and fibroblasts) was dominant in Control negative group (Figure 2 a and b), in comparison to treated group I that showed the late phase of granulation tissue and healing more progressed as in (Figure 2 c and d). While in Treatment groups of II and III, the healing became more pronounced and displayed an early stage of the re-epithelization phase, with forming immature-hyperplastic and disorganized epidermis surrounding the wound area with an increase in the thickness of the dermal layer by the presence of mature collagen fibers (Figure 3).

   b. Microscopical assessments of cutaneous wound healing on days eighteen:

   On day 21, after wounding, wound healing more progressed (proliferative phase) was apparent in Control negative group, epidermis formed as immature-hyperplastic and disorganized that overlying the area of the wound, the mild inflammatory reaction is seen in dermis and hypodermis with an increase in the thickness of dermal layer due to presence of well-organized collagen fibers as bundles with proliferative fibroblast (Figure 4 a and b). In Treatment group I, the wound thickness increased and covered by small scab and the wound showed the late stage of proliferation as in figure 4 c and d, tissue regenerated very well, while in Treatment groups II and III, the wound area covered by a normal epidermis and in the dermis, collagen fibers were thicker and denser. In the center of the wound the scar tissue was formed but in diverse levels in each group, for instance, in Treatment group II, new epidermis was formed with a mild thickness of keratinization, but marked density of scar tissue (granulation tissues beneath all wounds had matured to form scar tissues) were observed in the center of the wound, in dermal layer well organized thick bundles of collagen fibers was observed (Figure 5 a and b) if compared to the Treatment group III, that had well-uniformed epidermis with organized skin histological tissue layers encircle the wound area completely, with mild-moderate keratinization thickness, also, the moderate density of scar tissue and thick and well-arranged bundles of collagen fibers found in the dermis (Figure 5 c and d).
Figure 2: Histological sections of skin wound in Control negative group and Treatment group I showing granulation stage of healing wounds on day 8 following wounding. a and b: Presence of necrotic debris in the tissue surface with marked granulation tissue in the epidermis and dermal layers, epidermis, and dermis rich with newly formed granulation tissue, newly blood vessels (black arrows), and infiltration of inflammatory cells (PMN) as designated by red arrows, c and d: Thicken granulation tissue in dermis, neovascularization (black arrows), and intense infiltration of inflammatory cells as shown by red arrows, stained by H&E stain, (the scale bar of photo (a, c) 50µm, and (b, d) 20µm).
Figure 3: Histological sections of the cutaneous wound site in Treatment groups of II and III at day 8 following wounding showed the middle stage and the late stage of the proliferative phase respectively. b and b: Typical hyperplastic disorganized re-epithelialization formed for bridging the gap as indicated by red dash line, increased in the thickness of the dermal layer by a mature bundle of collagen fibers (black arrows); c and d: Hyperplastic-immature epithelium as designated by red dash lines with an improved in the thickness of the dermal layer by an extreme, mature bundle of collagen fibers (black arrows), stained by H&E stain, (the scale bar of photo (a, c) 50µm, and (b, d) 20µm).
Figure 4: Histological sections of skin wound in Control negative group and Treatment group I at day 21 following wounding. a and b: Immature disorganized epidermis (red dash line). Intense, mature bundle of collagen fibers increased the thickness of the dermal layer (black arrows) with proliferative fibroblasts as designated by yellow arrows. c and d: Small scab that contains fibrin and PMNs completely covered the wound area, epidermis formation (red dash line), thick compact and irregularly arranged collagen fibers as indicated by red arrows and insert. Few numbers of PMN that separate the wound from newly formed epidermis as in section d indicated by red dash line, stained by H&E stain, (the scale bar for photo (a, c) 50 μm, scale bar (b, d) 20 μm).
Figure 5: Histological skin wound section in Treatment groups of II and III at day 21 after wounding showed remodeling phase. a and b: Developing of the new epidermis with a mild-moderate keratinization thickness, the center of the wound contain the moderate-marked density of scar tissue (red dash lines) and proliferation of fibroblast (black arrows), well organized thick bundles of collagen fibers observed in the dermis. c and d: Developing of the new epidermis with a mild keratinization thickness, the center of the wound contain the marked density of scar tissue (red dash lines and black arrows), well structured, dense bundles of collagen fibers with inflammatory cell infiltration seen in the dermis as shown by black arrows, stained by H&E stain, (the scale bar of photo (a, c) 50µm, and (b,d) 20µm).

2. Hematological Study:

In this study, the hematological results of all treated mice shown in table 1; A significant mild-moderate increasing in the mean value of WBC ($P=0.02$), and Platelets ($P = 0.005$) were found in Treatment group II, as well as a decrease in the mean values of HGB ($P =0.01$), MCV ($P =0.01$) and MCH ($P =0.01$) also detected, while in Treatment group III, significant elevation only detected in the mean value of Platelets ($P =0.000$) besides, to decrease in MCH ($P =0.01$) and
MCHC ($P = 0.05$) mean values, additionally, the result revealed a significant decrease in the mean values of PCV% ($P = 0.01$) and MCH ($P = 0.000$) in Treatment group I, when compared with a control negative group. On the other hands the result of hematological analysis in table 1 showed non-significant effects in the following parameters in each groups; For instance in Treatment group II, there were an increase in RBC ($P = 0.68$), LYM ($P = 0.6$), and GRAN ($P = 0.67$) means, whereas in the same group PCV% ($P = 0.5$), MCHC ($P = 0.1$), and MPV ($P = 0.6$) showed non-significant reduction in there means, while in Treatment group III there was decrease in the means of RBC ($P = 0.06$), LYM ($P = 0.2$), HGB ($P = 0.16$), PCV% ($P = 0.36$), and MCV ($P = 0.3$) in addition to increase in WBC ($P = 0.7$), GRAN ($P = 0.52$), and MPV ($P = 0.5$) means, furthermore, there was non-significant decrease in the means of RBC ($P = 0.34$), MCV ($P = 0.1$), MPV ($P = 0.2$), and HGB ($P = 0.38$) besides elevation in the means of WBC ($P = 0.91$), LYM ($P = 0.3$), GRAN ($P = 0.32$), Platelet ($P = 0.2$), and MCHC ($P = 0.32$) in Treatment group I in comparison to the control negative group.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Control negative</th>
<th>Treatment I Lawsonia Inermis</th>
<th>Treatment group II Mustic Gum Resin</th>
<th>Treatment group III Quercus Brantii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RBC ($10^{12}$/L)</td>
<td>7.79±0.21</td>
<td>7.32±0.34</td>
<td>7.85±0.21</td>
<td>7.18±0.22</td>
</tr>
<tr>
<td>Total WBC ($10^{9}$/L)</td>
<td>3.34±0.21</td>
<td>3.40±0.26</td>
<td>4.92±0.28*</td>
<td>3.68±0.43</td>
</tr>
<tr>
<td>LYM ($10^{9}$/L)</td>
<td>2.92±0.49</td>
<td>3.56±0.41</td>
<td>3.56±0.41</td>
<td>2.50±0.41</td>
</tr>
<tr>
<td>GRAN ($10^{9}$/L)</td>
<td>0.52±0.08</td>
<td>0.61±0.13</td>
<td>0.65±0.11</td>
<td>0.65±0.12</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>12.94±0.24</td>
<td>10.87±0.74</td>
<td>10.24±0.10*</td>
<td>12.39±0.17</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>39.96±0.64</td>
<td>33.72±1.75**</td>
<td>38.98±0.99</td>
<td>38.93±0.74</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>52.58±0.59</td>
<td>50.56±1.31</td>
<td>48.79±0.81**</td>
<td>51.78±0.84</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>53.71±0.37</td>
<td>13.76±1.19***</td>
<td>15.42±0.45***</td>
<td>14.91±0.67***</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>32.38±0.23</td>
<td>32.98±0.37</td>
<td>31.52±0.47</td>
<td>31.36±0.52**</td>
</tr>
<tr>
<td>Platelets ($10^{9}$/L)</td>
<td>155.30±8.09</td>
<td>204.20±34.05</td>
<td>209.60±14.31***</td>
<td>258.38±17.02***</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>6.52±0.14</td>
<td>6.28±0.22</td>
<td>6.46±0.17</td>
<td>6.64±0.17</td>
</tr>
</tbody>
</table>

Within each row, values expressed by Mean±SE, values with small superscripts star vary from each other ** $P \leq 0.05$ and *** $P \leq 0.05$ vs. Control (n=10).
Discussion

In the current study we reported the wound healing potential of the Mustic Gum Resin (MGR), Quercus Brantii (QB), and Lawsonia Inermis, applied on wounds in BALB/c mice. MGR is a natural constituent and has many biological impacts such as analgesic, antioxidant, anti-inflammatory, and antimicrobial activities. Henna (LI), has antiseptic, anti-inflammatory and antimicrobial possessions. Along these lines, if any plant material has antimicrobial, pain-relieving, and anti-inflammatory effects together, this substance may likewise be required to help advance injury healing and lead to skin renewal. We observed that after 8 days of the topical application of Treatment group I (LI) enhanced late phase of granulation tissue and healing more progressed than in mice of control negative group. While in Treatment group II and III (MGR and QB) respectively, the healing became more pronounced and showed an early phase of the re-epithelization process.

The first reaction of wound healing is inflammation, which serves as a tissue protection mechanism that can withstand microbial contamination. Therefore, the significant wound healing activity of MGR and QB substances might be identified with its amazing anti-inflammatory impact. For the injury repairing period, the antimicrobial effort is significant because the injury that is presented to the external circumstance is progressively powerless against microbial assaults which ordinarily lead to delays in the healing procedure. Threat factors like pathogens can thus jeopardize the repair process. The most common pathogen responsible for infection of skin wounds is S. aureus and P. aeruginosa.

Complete wound healing seen in 21 days post-operation more specifically in Treatment group II and III (MGR and QB) respectively, the histological findings showed normal epidermis covered the wound area and unique tissue recovery was a lot more prominent in skin wounds rewarded with Treatment group II and III (MGR and QB) correspondingly, than in wounds of control negative and even Treatment group II (LI). The wound healing effects of (MGR) fruit oil have previously been studied in a rabbit burn model and its ability to increase in re-epithelialization has been established and it’s in agreement with our study. The oil exhibited improved wound healing efficacy, which could be attributable to the synergistic effect of all the components found in Pistacia lentiscus oil, especially fatty acids, tocopherols, and sterols. Such phytochemicals showed desirable wound healing functions and beneficial behaviors through different methods at the various stages of the wound healing cycle involving antimicrobial, anti-inflammatory, antioxidant, collagen synthesis enhancement, cell proliferative and angiogenic impact.

In accordance to our results, other studies proposed that the oak can enhance the angiogenesis phase, reepithelialization and wound recovery process in male adult albino rats. Because of the antimicrobial, antioxidant, and inflammatory effects of most plant extracts, wound healing can aid.

Some research reported the influence of henna on wound healing, which is compatible with the recent report, suggesting that henna’s antibiotic and anti-inflammatory properties are generally effective in wound healing, also demonstrated that henna extract decreased the mean cutaneous wound diameter and improved dramatically healing up to 12 days.

The contraction of wounds is facilitated by specialist myofibroblasts located in the granulated tissue. The increase in wound contraction in in mice of Treatment group II and III (MGR and QB) correspondingly, in our finding might be a result of the enhanced activity of fibroblasts and agrees with the previous report, who has shown that the injury reaction includes the proliferation and migration of cells such as fibroblasts, endothelial and epithelial cells and the deposition of connective tissue and wound contraction.

The results of the hematological evaluation of the blood samples in table 1 showed significant changes in the following parameters; For example in the Treatment group II (MGR) increasing in the mean value of WBC these discoveries associate very well with a past report, this was overwhelmingly accounted by an intensification in the circulating neutrophil infiltration, perhaps as a result of recruitment or demarcation of neutrophils from bone marrow. Also in the MGR treated group there was a significant increase in the mean value of blood platelets count, in agreements with former studies that found the increase in megakaryocytes may lead to increased blood platelets, or platelet production can increase due to inflammatory disease. As well as a slight decrease in the mean of HGB, MCV, and MCH which is corroborated similar findings reported by in mice. While in Treatment group III (QB) significant elevation only detected in the mean value of Platelets, besides, to decrease in MCHC and MCH mean values which is in agreements with that found by. The
decrease in the MCHC value that results from the toxic impact on red blood cell count and hemoglobin concentration of these natural ingredients, as the validity of these indexes is determined by the red cell count, hemoglobin concentration and packaged cell volume values. Additionally, the result revealed a significant decrease in the mean values of PCV% and MCH in Treatment group I (LI) treated group when compared with control negative group similarly decrease in PCV was observed by, which can be disclosed as because of a huge reduction in Hb, this would prompt reduction in the size of RBCs and thus the last decline in PCV. PCV regards are likewise noteworthy in estimating anxiety on animal health and demonstrate the limit of the blood to convey oxygen. Reducing the WBC and PCV values in the treated mice indicates stress.

**Conclusion**

The present study demonstrated that the Mastic Gum Resin, Lawsonia Inermis, and Quercus Brantii plant extracts were more effective in wound healing when used for 21 days after incision; further studies will also be required to study these effects and their mechanism of action in detail.

**Conflict of Interest:** Nil

**Source of Funding:** Self-funding

**References**


Clinical and Histopathological study of black and Red Grape Seed extracts (\textit{Vitis Vinifera}) effects on the Albino Mice

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Abstract

Grape seed extract (GSE) is a complex mixture of several compounds, mostly represented by polyphenols and phenolic acids. The goal of the pilot study was to illustrate the safe dose of GSE in mice model and to assess toxicity that may be initiated by the different concentrations of this plant. Forty-two mice were divided equally into 7 groups; groups 1 attended as control, were only received water, whereas animals of groups 2, 3, 4 were treated orally with 200, 400, and 800 mg/kg b.w. of black grape seed extract respectively, and the remaining groups including 5, 6, and 7 were treated orally with 200, 400, and 800 mg/kg b.w. of red grape seed extract respectively. The animals were observed daily for any sign revealing for activity alterations and toxicity along with their body weight measurement during the experiment for 21 days with histopathological examination. The results gained from this pilot study were recording that the 200 and 400 mg/kg b.w. doses of GSE were safe compared to the 800 mg/kg b.w. in both black and red grape seed extracts because the higher dose led to a reduction in body weight gain and produce changes in the mentioned organs.

Keywords: Albino mice, Black grape seed, Histopathology, Red grape seed, Sharbazher village.

Introduction

Plants can produce a large number of chemical compounds with significant biological effects and they have been used to manufacture numerous kinds of medicines since the creation of human being ¹,²

Grapes, \textit{Vitis vinifera} berries, consider as conventional valued fruits in the world ³. The primary composition of grape pomace is skin, steam, and seeds ⁴. The fresh grape chemical composition is nearly 70-80% water and dissolved solids such as sugars, phenolic compounds, nitrogenous compounds, organic acids, minerals, aroma compounds, pectic substances ⁵. A variety of bioactive compounds such as simple phenolics, flavonoids, anthocyanins, stilbenes, proanthocyanidins, and vitamin E are considered a distinctive mixture of phytochemicals in grapes ⁶. Grape seeds consider as a waste product in industry and consist of protein, carbohydrates, lipids, and 5-8% of polyphenols reliant on the type of grapes ⁷. Different cultivars have a different grape seed composition ⁸. Additionally, revenues and seed quality affected by several environmental and biological factors, such as light, drought, high salinity, cold, metal ions, pollutants, xenobiotics, toxins, experimental manipulations, pathogenic infection, and aging of plants ⁹. A multitude of flavonoids is contained in GSE ¹⁰. The most abundant of these are the proanthocyanidins, which are oligomers of monomeric flavan-3-of units linked by carbon-carbon bonds ¹¹.

The most plentiful biologically active phytonutrients among the polyphenols found in grapes are flavonoids, which are possessing cardioprotective, neuroprotective, antimicrobial, anti-aging, antioxidant, anti-inflammatory, and anti-cancer properties ¹²,¹³.

Oral toxicity studies dealing with grape seed safety in experimental animals are few in the Iraq/Kurdistan region. Therefore, the main objective of this pilot study was to determine the oral toxicity of acetone-extracted
grape seed (black and red) extract at different doses in mice, through clinical observations and evaluation of histopathological changes in multiple organs.

**Materials and methods**

**Grape seed sampling and extraction**

The work was carried out on two different varieties of grape (*Vitis Vinifera*) the red and black, which were taken manually from Sulaimani (Sharbazher-Kurdistan region) in the middle of July. The grapes were isolated manually from the skin, dried in the open air away from direct sunlight, and grounded into powder by the electrical grinder. The powders were stored in dark glass containers at -20°C until the use.

Each sample of (72.2 g) of red grape seed and (40.73 g) of black grape seed powders was suspended with 202 mL and 114 mL of (70%) aqueous acetone respectively in a 500 mL Erlenmeyer flask. The mixtures were left on a magnetic stirrer for 24 hours at room temperature. The resultant extracts were filtered on a Buchner funnel then firstly evaporated with a rotatory evaporator to remove acetone, finally were freeze-dried for 24 hours to remove the water.

**GC–MS analysis**

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to ascertain the compounds present in the purified samples of the red and black types of grape seed (*Vitis Vinifera*) from Sulaimania (Sharbazhear-Kurdistan region). Our natural plant was evaluated by (GCMS-QP2010 Ultra) GC systems combined with a mass spectrometer.

Animals and grape seed extract treatments

Forty-two mice (Male and female *Mus Muscular* species, *BALB/c* strain) weighing 25-30 g, at 4 weeks age were purchased from the Animal House at the College of Veterinary Medicine, University of Sulaimani (Sulaimani, Iraq/Kurdistan region), the mice were provided tap water and standard food *ad libitum* and were permitted to acclimate for one week before the start of the experiment accommodated in temperature and light-controlled environment. All of the in vivo experimentation in this pilot study was performed humanely according to and the ethical approval that was obtained from the Ethics Committee at the College of Veterinary Medicine, the University of Sulaimani in number (01589).

Consequently, the mice were assigned into 7 groups (6 mice per group) as follows: Group 1 (control): the animal of which were received normal saline without treatment; Group 2, 3, and 4, the animal of which treated orally with 200, 400, and 800 mg/kg b.w. of black GSE, respectively; Group 5, 6, and 7, the animal of which were treated orally with 200, 400, and 800 mg/kg b.w. of red GSE respectively. All treatments were given as a single daily dose by oral gavages every two days with a third-day free treatment and this study continued for about 21 days.

**Clinical observations and body weight measurements**

The animals were examined daily during this pilot study (for 21 days) for signs of acute toxicity such as diarrhea, curved tail, falling of hair, mortality and any other sign indicative for activity alterations, and the body weights of mice were recorded 2 times throughout the pilot study (day 0 and at the third week).

**Tissue sampling and histopathological examination**

At the end of the experimental period, the mice were euthanized with (Xylazine-Ketamine: 0.1 mL/10 gm of body weight) as recommended dose intraperitoneally and cervical dislocation. The liver, kidney, spleen, and lung tissues of the sacrificed mice were excised, cleaned by normal saline, cut into the 4mm, fixed in 10% neutral buffered formalin (PH 7.6) for 24 hours and underwent a series of histopathological processes, tissue slices of 4 μm thick were attained and stained using the standard H and E technique and envisioned by light microscope (Leica, Germany), connected with an image analyzer software (Am Scope, AmView, MU1000B).

**Statistical Analysis**

Statistical analysis was performed using the ANOVA (One-way) analyses of variance. Results were presented as a mean±standard error (Mean±SE) and *P* values less than 0.05 were considered significant. All statistical explorations were accomplished using the SPSS software version 22 (SPSS Inc., USA).

**Results**

Gas chromatography-MASS Spectroscopy

The secondary metabolites present in red and black grape seeds were detected in GC-MS (Table 1and 2).
The Grape Seed (GSD) peak region percentage and peak area coverage are specified below.

### Table 1: G-CMS components for red grape seed

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>RT (Min.)</th>
<th>Compound Name</th>
<th>Peak Area</th>
<th>Correct area</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.439</td>
<td>Heptane, 3-methyl</td>
<td>4280</td>
<td>562353</td>
<td>19.620%</td>
</tr>
<tr>
<td>2</td>
<td>3.808</td>
<td>Octane</td>
<td>9767</td>
<td>1039837</td>
<td>36.279%</td>
</tr>
<tr>
<td>3</td>
<td>7.345</td>
<td>2-N-PROPYL-1-D1-AZIRIDINE</td>
<td>5538</td>
<td>168753</td>
<td>5.888%</td>
</tr>
<tr>
<td>4</td>
<td>11.304</td>
<td>Tridecane, 2-methyl</td>
<td>4642</td>
<td>87857</td>
<td>3.065%</td>
</tr>
<tr>
<td>5</td>
<td>14.960</td>
<td>Eicosane, 2-methyl</td>
<td>3572</td>
<td>66809</td>
<td>2.331%</td>
</tr>
<tr>
<td>6</td>
<td>18.239</td>
<td>Cyclobutanone, oxime</td>
<td>3308</td>
<td>68064</td>
<td>2.375%</td>
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<tr>
<td>7</td>
<td>19.749</td>
<td>Di-isodecyl phthalate</td>
<td>5223</td>
<td>365445</td>
<td>12.750%</td>
</tr>
<tr>
<td>8</td>
<td>21.187</td>
<td>Octane, 2,4,6-trimethyl-</td>
<td>4653</td>
<td>93120</td>
<td>3.249%</td>
</tr>
<tr>
<td>9</td>
<td>22.193</td>
<td>1,2-Benzenedicarboxylic acid, bis 2-methylpropyl ester</td>
<td>9067</td>
<td>212306</td>
<td>7.407%</td>
</tr>
<tr>
<td>10</td>
<td>22.554</td>
<td>4,4-Dimethylcyclooctene</td>
<td>3692</td>
<td>76959</td>
<td>2.685%</td>
</tr>
<tr>
<td>11</td>
<td>23.444</td>
<td>2-Acetyl-N-methylaniline</td>
<td>2537</td>
<td>58464</td>
<td>2.040%</td>
</tr>
<tr>
<td>12</td>
<td>23.856</td>
<td>Docosane</td>
<td>2972</td>
<td>66227</td>
<td>2.311%</td>
</tr>
</tbody>
</table>

### Table 2: G-CMS components for black grape seed

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>RT (Min.)</th>
<th>Compound Name</th>
<th>Peak Area</th>
<th>Correct area</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.445</td>
<td>Heptane, 3-methyl</td>
<td>4267</td>
<td>579464</td>
<td>3.736%</td>
</tr>
<tr>
<td>2</td>
<td>3.822</td>
<td>Octane</td>
<td>9823</td>
<td>998433</td>
<td>6.438%</td>
</tr>
<tr>
<td>3</td>
<td>7.349</td>
<td>2-N-PROPYL-1-D1-AZIRIDINE</td>
<td>5775</td>
<td>161076</td>
<td>1.039%</td>
</tr>
<tr>
<td>4</td>
<td>11.305</td>
<td>Heptadecane, 2-methyl-</td>
<td>5492</td>
<td>106189</td>
<td>0.685%</td>
</tr>
<tr>
<td>5</td>
<td>14.961</td>
<td>Hexadecane, 2-methyl-</td>
<td>4376</td>
<td>85425</td>
<td>0.551%</td>
</tr>
<tr>
<td>6</td>
<td>25.428</td>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>115510</td>
<td>7027490</td>
<td>45.312%</td>
</tr>
<tr>
<td>7</td>
<td>26.876</td>
<td>Di-isodecyl phthalate</td>
<td>140559</td>
<td>6550999</td>
<td>42.240%</td>
</tr>
</tbody>
</table>
**Clinical observations**

The animals were healthy in general with no clinical signs of toxicity. There were no unusual changes in behavior or locomotor activity during the 21-day observation period. No deaths occurred during the study.

**Body weight measurements**

All groups gained weight during the pilot study period compared to day 0 of the study. The mean body weight of the mice of all groups showed a significant increase ($P<0.05$) in their body weight measurements in comparison to the initial day, no significant variations ($P>0.05$) were seen in the mean of the body weight gain of mice in the treatment groups of 200 mg/kg b.w. and 400 mg/kg b.w. in comparison with that of the control groups. While significant reduction ($P<0.05$) in the mean body weights gain was recorded for 800 mg/kg b.w. groups in the last week of an experiment in comparison with that of the mice in the control and other treated groups (Table 3 and 4).

<table>
<thead>
<tr>
<th>Table 3: Body weight gain (gram) of mice in black GSE treated groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A black grape seed extract</strong></td>
</tr>
<tr>
<td>Experiment duration</td>
</tr>
<tr>
<td>Day 0</td>
</tr>
<tr>
<td>Week 3</td>
</tr>
<tr>
<td>Weight gain</td>
</tr>
</tbody>
</table>

The body weight gains are expressed by mean ± standard error, bodyweight values, values marked by different letters are significantly different ($p<0.05$).

<table>
<thead>
<tr>
<th>Table 4: Bodyweight gain (gram) of mice in red GSE treated groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A red grape seed extract</strong></td>
</tr>
<tr>
<td>Experiment duration</td>
</tr>
<tr>
<td>Day 0</td>
</tr>
<tr>
<td>Week 3</td>
</tr>
<tr>
<td>Weight gain</td>
</tr>
</tbody>
</table>

The body weight gains are expressed by mean ± standard error, bodyweight values, values marked by different letters are significantly different ($p<0.05$).
Histopathological Results

Regarding histopathological findings, the liver section of control, and 200 mg/kg b.w. treated groups in the black and red GSE exhibited normal histomorphological features including, central vein, sinusoidal capillaries with normal appearance of hepatocytes (Figure 1a-d), in comparison to 400mg/kg b.w. treated groups in the black and red GSE, the liver cells showed karyolitic features (Figure 1e and f), while the hepatocytes in 800mg/kg b.w. treated groups in both types of GSE undergo coagulative necrosis, characterized by eosinophilic cytoplasm with features of karyolysis and karyorrhexis of the nucleus and kupffer cell proliferation (Figure 1g and h). The microscopical section of the kidney in the control group showed; normal and intact appearance of glomeruli, proximal and distal convoluted tubules with normal renal vasculature in control, and 200mg/kg b.w. in black and red GSE treated groups (Figure 2a-d). While in 400 mg/kg b.w. in black and red GSE treated groups the epithelial lining of collecting tubules showed slightly swollen. In the 800mg/kg b.w. in both types of GSE treated groups, the kidney showed dilation of Bowman’s capsule, glomerular atrophy with the segmentation of glomerular capillaries and increasing the mesangial cells, also moderate swollen of the epithelial lining of convoluted tubules with interstitial hemorrhage (Figure 2e-h). Microscopical section of the spleen revealed normal histological appearance in control and 200mg/kg b.w. in black and red GSE treated groups, whereas in 400mg/kg b.w. in both types of GSE treated groups showed mild-moderate lymphocytic hyperplasia in the white pulp region and congestion in the red pulp area if compared to the 800mg/kg b.w. in black and red GSE treated groups that showed moderate lymphocytic hyperplasia in white pulp region and congestion in the red pulp area (Figure 3a-h and 6a-h). The histopathological finding of lung parenchyma in black and red GSE treated groups revealed normal histological structures of bronchi, bronchioles, alveolar ducts, alveolar sac, and alveoli with normal vasculatures in control and 200mg/kg b.w. treated groups, but the minimum-mild proteinous fluid was found in the alveolar lumen of 400 and 800mg/kg b.w. black and red GSE treated groups in addition to vascular congestion particularly in the 800mg/kg b.w. black and red GSE treated groups.

Figure 1- histomicrograph of liver sections in control, black and red GSE treated groups. a and b: The normal liver histology in the control group, c and d: The normal histological features of liver parenchyma in 200mg/kg b.w. treated groups, e, and f: The hepatocytes showed slightly swollen (black arrows) with karyolysis in few hepatocytes (yellow arrows) in 400mg/kg b.w. treated groups, g and h: The hepatic cells undergo coagulative necrosis as indicated by black arrows in 800mg/kg b.w. treated groups, (S) sinusoidal capillaries, (H&E stain, scale bar 50 μm, scale bar 20 μm).
Figure 2- Histomicrograph of kidney sections in control, black and red GSE extract treated groups. a and b: The normal kidney histology in the control group, c and d: The normal histological structures of kidney parenchyma in 200mg/kg b.w. treated groups, e, and f: The epithelial lining of PCT and DCT showed slightly swollen (black arrows) in 400mg/kg b.w. treated groups, g and h: The swollen of the Bowmans’ space (blackhead arrows), segmentation of glomerular capillary tuft with mesangial hypercellularity and glomerular atrophy, the epithelial lining of PCT and DCT showed moderately swollen (black arrows) with interstitial hemorrhage indicated by yellow arrows in 800mg/kg b.w. treated groups, (DCT) convoluted tubules, and (DCT) distal convoluted tubules, (H&E stain, scale bar 50 μm, scale bar 20 μm).

Figure 3- Histomicrograph of spleen parenchyma in control, black and red GSE treated groups. a and b: The normal spleen structures in the control group, c and d: Normal microscopical features of the spleen in 200mg/kg b.w. the treated groups, e, and f: Slightly lymphocytic hyperplasia (black arrows) in the white pulp regions (black arrows) in 400mg/kg b.w. treated groups, g and h: Mild-moderate lymphocytic hyperplasia (black arrows) in the white pulp regions and congestion of the red pulp sinusoids as indicated by blackhead arrows in 800mg/kg b.w. treated groups, (WP) white pulp, and (RP) red pulp, (H&E stain, scale bar 50 μm, scale bar 20 μm).
Discussion

For the continuous development of phytochemicals under-regulated plant cell cultures, prospective replacements are generally considered 18,19. In recent years, plant cell biotechnology of grapes and particularly of grape cell suspensions have enjoyed great scientific and industrial consideration 20. New research aims to decode the benefits of grapes as a rich source of essential phytoneutrients with remarkable beneficial impacts on human health 21.

No mortality was reported with no significant changes in body weight gain or physical appearance, and no abnormal histopathological changes in the liver, kidney, spleen, and lung were observed during this pilot study in that groups received the 200, and 400mg/kg b.w. doses of both black and red GSE and the outcomes attained from this study are, in agreement with the results of other studies that revealed using of the GSE in experimental animals did not result in any disturbances in clinical activities or body weight loss 22,23.

However, the significant decrease in body weight gain was found in mice of group 800mg/kg b.w. of GSE, Yamakoshi, et al, (2002), who reported a similar result in which the high dose of GSE may lead to slightly significant weight loss in an animal model 24, while the observed result may disagree with the Mittal et al, (2003), study who documented that the high dose of GSE did not interfere with physical activity and significant difference in body weights or other signs of clinical toxicity 25. Also, the other study on Sprague-Dawley rats documented no significant decrease in body weight was observed even after 90 days of oral administration of GSE 26. The decrease in body weight gain may correlate with the high antioxidant activity in induced to enhance the lipase effect and enhancing lipolysis 27.
Additionally, it is obvious from the present study the histopathological changes in 800mg/kg b.w. dose groups of GSE represented by the mild-moderate swollen in the hepatocytes with a moderate degree of necrosis, segmentation of glomerular capillaries and moderate swollen of the epithelial lining of convoluted tubules with interstitial hemorrhage in kidney organ, the histological changes in spleen revealed mild-moderate lymphocytic hyperplasia in the white pulp region and congestion in the red pulp area, and the lung parenchyma showed mild accumulation of eosinophilic fluid the alveolar lumen. Our findings disagree with the other studies that mentioned no significant histopathological lesions in multiple organs after administration of GSE high dose \(^{28,29}\).

**Conclusion**

Consequently, it is concluded that the results of this study support the health of GSE dietary components for human use. No observed level of adverse effects (NOAEL) was deemed approximately in a dose of 400 mg/kg b. w. /day for administration in both types of GSE. While a mild-moderate changes microscopically was seen in the mice’s organs that treated by 800mg/kg b.w. black and red GSE treated groups.

**Conflict of Interest:** Nil

**Source of Funding:** Self-funding

**References**

16. Hassan SM, Remzi DO, Muhammed SF, et al. Photo Protective Role of Wild Edible Plants on Skin of Mice from Harmful Effects of Ultraviolet


Detection of Pork in Canned Meat Products by using DNA-based Methods

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Abstract

Adulteration of meat products became a matter of great concerns of religious, economical, legal and hygienic aspects. Canned meat is one of the most favorable in a lot of countries, which makes it prone to adulteration. The objective of the current study was to identify pork in canned meat for the presence of adulteration in commercial market of Basrah city/Iraq. Thirty canned meat were collected from commercial market. The possibility of a species mixture was tested with polymerase chain reaction (PCR), targeting pork (290bp). Analysis of canned meat revealed negativity results of all samples to pork meat. In conclusion, samples analysed in the current study showed that there was no adulteration by mixing pork meat in canned meat products due to absent production of pork flesh for religion and hygienic aspects. Beef and mutton flesh might be replaced in chicken, horse, and donkey flesh for economic reason.

Key words: Canned meat, fraud, meat species identification, mislabeling, PCR.

Introduction

Meat is the muscle tissue of an animal that is consumed as food. It is composed of high amount of water (75%), protein (20%), fat (5%) and small amount of carbohydrates, vitamins, and minerals. Meat products are considered a favorite item in a lot of countries and due to the consumption of meat products continues to elevate, mislabeling and adulteration of meat products have become common. Consumers usually rely on food labeling to make right choices for religious and public health reasons. The success this kind of adulteration in processed meat is invisible changes in the appearance, color, texture, and flavor of the processed meats. Under food labeling regulations, adulteration by mixing or replacement undeclared species in meat products is illegal. Recently, detecting the meat species in meat product is considerable importance issue to ensure the food safety for public health.

Methods have been used to detect species of meat are based on either protein or DNA detections. The protein detection techniques are unable to identify between close relatives species. In addition, these techniques are required complicated isolation procedure and time consuming. However, DNA detection technique is considered as reliable, efficient, simple and a quick method to identify meat species. In previous studies, testing processed of meat products by DNA detection technique revealed the use of a label that is incorrect. Since becoming aware of these issues, this work aimed to identify pork in canned meat products by detection the meat specie in it under laboratory conditions.

Materials and Method

2.1 Sample collection

A total of 30 sample (3 canned meats from each product) were collected from various commercial markets in Basrah city/ Iraq. These samples including products labeled as beef luncheon meat (Baidar, Kingdom of Saudi Arabia), beef luncheon meat (Hena, United Arab Emirates), beef luncheon meat (Ghadeer, Jordan), corned beef loaf (Burdon, Brazil), beef hot dog (AlTaghziah, Lebanon), beef hot dog (Al Qaisar, Kingdom of Saudi Arabia), chicken luncheon meat (Hena, United Arab Emirates), chicken luncheon meat (Baidar, Kingdom of Saudi Arabia), chicken luncheon meat (Alatyab, Turkey), and chicken hot dog (AlTaghziah, Lebanon). Following collection, samples were kept at room temperature until analysed. The collected samples were marked numerically (Table 1).
## Table 1: Canned meat products analysed for authentication

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Product label</th>
<th>Trademark</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beef Luncheon Meat</td>
<td>Baidar</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
<tr>
<td>2</td>
<td>Beef Luncheon Meat</td>
<td>Hena</td>
<td>United Arab Emirates</td>
</tr>
<tr>
<td>3</td>
<td>Beef Luncheon Meat</td>
<td>Ghadeer</td>
<td>Jordan</td>
</tr>
<tr>
<td>4</td>
<td>Corned Beef Loaf</td>
<td>Burdon</td>
<td>Brazil</td>
</tr>
<tr>
<td>5</td>
<td>Beef Hot Dog</td>
<td>AlTaghziah</td>
<td>Lebanon</td>
</tr>
<tr>
<td>6</td>
<td>Beef Hot Dog</td>
<td>Al Qaisar</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
<tr>
<td>7</td>
<td>Chicken Luncheon Meat</td>
<td>Hena</td>
<td>United Arab Emirates</td>
</tr>
<tr>
<td>8</td>
<td>Chicken Luncheon Meat</td>
<td>Baidar</td>
<td>Kingdom of Saudi Arabia</td>
</tr>
<tr>
<td>9</td>
<td>Chicken Luncheon Meat</td>
<td>Alatyab</td>
<td>Turkey</td>
</tr>
<tr>
<td>10</td>
<td>Chicken Hot Dog</td>
<td>AlTaghziah</td>
<td>Lebanon</td>
</tr>
</tbody>
</table>

2.2 Sample Preparation for DNA extraction

The sample (2 g) was aseptically collected using sterile forceps, cut using a sterile scalpel, and mixed thoroughly with distal water (60 ml) in a blender (230 rpm for 120 sec) to homogenize (Figure 1). The sample was then transferred into two microcentrifuge tube (1.5 ml). To prevent DNA degradation, samples stored at -20 °C until analysis.

2.3 DNA extraction and detection

DNA extraction was carried out for all canned meat samples in triplicate using the DNA extraction kit GsyncTMDNA (Geneaid Biotech Ltd., Taiwan). The extraction procedure was done according to the manufacturer’s instruction. Briefly, the tissue sample (25 mg) was lysed with ATL buffer (200 μL) and Proteinase K (20 μl), vortexed at 30 min, and incubated overnight at 60 °C. Then, GSB buffer (200 μl) and absolute ethanol (200 μl) was added and vortexed (10 sec). The sample was then transferred to GC columns, washed with W1 buffer (400 μl), centrifuged (14,000 xg for 30 sec), followed by second washed with W2 buffer (600 μl), centrifuged (16,000 xg for 30 sec), and discard the flow through. To dry the column matrix, the GC column centrifuged at 16,000 xg for 3 min. After that, preheated elution buffer (100 μl) was added, stand at room temperature for 3 min to allow elution buffer, and finally centrifuged (16,000 xg for 30 sec) to elute purified DNA. DNA quantity was determined by using a Nano-Drop 2000 spectrophotometer (Nano Drop Technologies, Wilmington, USA).

2.4 PCR primers and amplification

The primers described in previous study were used to amplify a 12S rRNA region (Table 2).
Table 2: Oligonucleotide Primer sequence and target fragment for PCR assay

<table>
<thead>
<tr>
<th>Species</th>
<th>primer sequence</th>
<th>Target fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork</td>
<td>5'-CTACATAAGAATATCCACCACA-3' 5'-ACATTGTGGGATCTTCTAGGT-3'</td>
<td>290 bp</td>
</tr>
<tr>
<td>Chicken</td>
<td>5'-TGAGAACTACGAGCACAACAC-3'</td>
<td>183 bp</td>
</tr>
</tbody>
</table>

2.5 Polymerase Chain Reaction (PCR) assay for gene amplification

DNA from the samples was amplified in a total 25 μL reaction volume containing genomic DNA (1μg) of each species, primers (1μM), MgCl₂ (2mM), dNTP (0.2mM), PCR buffer (2.5μL of 10X) and the enzyme Taq DNA polymerase (1 unit). The Polymerase chain reaction assay conditions were performed by thermal cycler as follows: initial denaturation step (94°C for 4 min) followed by 30 cycles of: denaturation step (94°C for 30 sec), annealing step (57-64°C for 30 sec), extension step (72°C for 30 sec), and a final elongation (72°C for 30 sec) 10.

2.6 PCR products detection

The PCR production was detected on agarose gels (1.5%) prepared with agarose in Tris-borate-EDTA buffer (1x) at 100 V for 30 min, stained with a fluorescent stain (Ethidium Bromide) and images by gel-documentation systems (UViDoc UK). The size of the band was determined by comparison with a standard DNA ladder 10.

Results and discussion

Under food labeling regulations, adulteration by mixing undeclared species in meat products is illegal. Recently, detecting the meat species in meat products is an important issue for public health. A target fragment of pork (290 bp) was amplified. PCR analysis of canned meat revealed negativity results of all samples for pork meat (Table 3)(Figure 2). This find is in agreement with previous studies, in which there was no adulteration by mixing pork meat in meat products 10, 11, 12. It has been found that pork meat replacement in some countries is rarely due to absent production of pork flesh for halal food status. However, the tendency to mix pork meat and fat into the processed meats is more frequent in other countries due to high production of pork flesh and its cheapness. The previous study found that meat products that were declared as buffalo contained pork 7, 13. In addition, beef and mutton meat might replace in chicken, horse, and donkey meat. In Turkey, meat products (uncooked beef burger, kofta, sausage, and luncheon) that were declared as beef contained poultry and donkey meat 10. In addition, raw meat that was declared as beef contained mix of horse and deer meat.

![Analysis the DNA using PCR system](image1)

![Gel electrophoresis and results analysis](image2)
In China, meat products that were declared as buffalo contained cattle, pork, and duck meat. In Iran, raw burgers that were declared as beef contained poultry. One of the reasons for the replacement of chicken, horse, and donkey flesh in beef and mutton flesh is economic. The success of this kind of adulteration in processed meats is invisible changes in the visual inspection (appearance, color, texture, and flavor) of the processed meats.

### Table 3: PCR results for canned meat samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Product label</th>
<th>Adulteration ingredients PCR results (Pork)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beef Luncheon Meat/ Baidar</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Beef Luncheon Meat/ Hena</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Beef Luncheon Meat/ Ghadeer</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Corned Beef Loaf/ Burdon</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Beef Hot Dog/ AlTaghziah</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Beef Hot Dog/ Al Qaisar</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Chicken Luncheon Meat/ Hena</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Chicken Luncheon Meat/ Baidar</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Chicken Luncheon Meat/ Alatyab</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Chicken Hot Dog/ AlTaghziah</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (+) denotes for presence and (-) stands absence.

Figure 2: PCR product with 290 bp using pork specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negativity result of all samples for regarding to pork meat (L1-L10). M: molecular marker (100 bp ladder); lane 1: beef luncheon meat (Baidar, Kingdom of Saudi Arabia); lane 2: beef luncheon meat (Hena, United Arab Emirates); lane 3: beef luncheon meat (Ghadeer, Jordan); lane 4: corned beef loaf (Burdon, Brazil); lane 5: beef hot dog (AlTaghziah, Lebanon); lane 6: beef hot dog (Al Qaisar, Kingdom of Saudi Arabia); lane 7: chicken luncheon meat (Hena, United Arab Emirates); lane 8: chicken luncheon meat (Baidar, Kingdom of Saudi Arabia); lane 9: chicken luncheon meat (Alatyab, Turkey); lane 10: chicken hot dog (AlTaghziah, Lebanon).

**Conclusion**, samples analysed in the current study revealed that there was no adulteration by mixing pork meat in canned meat products. Pork meat replacement in some countries is rarely due to absent production of pork flesh for halal food status. However, Beef and mutton flesh (more expensive) might be replaced in chicken, horse, and donkey flesh (cheaper). To allow the consumer to make right choices when purchasing canned meat, canned meat must be frequently analysed using effective methods by governmental institutions.

**Acknowledgements**

The authors thank the Veterinary Medicine College, Basrah University for offering support to achieve this research. The authors also thank all staff and technicians of the Department of veterinary Public Health for their kindness and useful advices.

**Conflict of Interest**: Nil
Source of Funding: Self-funding

Ethical Clearance: Taken from the Scientific Committee, University of Basrah.

References


Effects of Adding Dry Mulberry Leaves (Morusalba) in the Concentrate Diet on Digestibility and Some Blood Parameters in Female Goats

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¹Assist. Prof., ²Assist. Lecturer, College of Veterinary Medicine, University of Baghdad, Iraq

Abstract

This experimental study was conducted at the animal farm of the College of Veterinary Medicine of Baghdad University during the period 15/3/2019 until 30/4/2019. Fourteen female local breed goats, at aged between 1.5–2 years and range weight 23–27kg. The animals were divided into two equal groups as following G1(control) was fed on concentrate diet 2% B.W and alfa - alfa hay. G2 group was fed the same diet and 2% grinding Mulberry leaves of concentrate diet. water was offered freely. For evaluation some animal performance. Bodyweight, some nutrients digestibility, blood picture and biochemical parameters. Results revealed that an excellent improvement was observed in body weight and nutrients digestibility were significantly (P<0.05). Similar effects in blood biochemical parameters (total protein and urea), while no differences were determined in triglycerides and cholesterol. Other blood parameters showed that hemoglobin (Hb) and packed cell volume (PCV) which were significantly higher (P <0.05) and further improved in erythrocytes, leucocytes, lymphocytes and neutrophils in the group received Mulberry leaves than control groups.

Keywords: goat, Mulberry leaves, cholesterol, performance, digestibility, leucocytes

Introduction

Livestock, mainly goats and sheep, rearing are a mainstay in many countries and most of the rural population depends on livestock and them by-products¹. The lack of feedstuffs and their high prices, especially in dry seasons, are among the most important difficulties that stand in front of ruminants’ breeders²,³. Livestock have the ability to respond to the different feed additives that improve their overall performance by improving the efficiency of utilizing nutritional content and reducing the risk of metabolism⁴. There are a number of non-food additives, such as antibiotics, they are improve the performance of the animal, but they have a number of side effects due to their toxicity the rumen microorganisms. In addition to their sedimentation inside living tissue cells and the transmission to their products⁵. This led to the searcher for sources for other natural food additives such as Mulberry tree leaves, which are distinguished by their high ability to produce green leaves containing different nutrients⁶. High the protein content with slightly dissolving in the rumen and soluble carbohydrates⁷,⁸. In addition to being rich in sulfur and other mineral elements and they are free of toxic substances, they are highly palatable by ruminant animals⁹,¹⁰. It isfurther; contribute to improve the digestion nutrients, rumen fermentation by improving the internal environment of the rumen¹¹. Flavonoids, the most important were isolated from the leaves of Mulberries which have nine flavonoids¹². It was found when adding the Mours leaves to the diets lead to improve the overall animals performance, increases the feed intake improved digestion, absorption, growth and development of the mammary glands and improve the immune functions of the body¹³,¹⁴. Also it, could be used fresh or dry berries leaves as natural food additives with concentrated or roughages in poor-quality feed-in ruminants¹⁵. For that, this study was conducted to evaluate the effect of adding Mours leaves to the concentrated diet on the performance and some blood parameters in female goats.
Materials and Methods

Animals and diets.

This study was carried out from 15/March till 30/April, 2019 at the animal farm of College Veterinary / University of Baghdad. Total animals, fourteen female local bred goats, aged 1.5-2 years and weight range 23-27kg. The animals were divided into two equal groups . Group 1(G1) received concentrate diet by 2%B.Was the control. Group 2(G2) received concentrate diet (2%B.W) contain 2% Mulberry leaves dry (grinding), each group had water ad libitum and green grass while the concentrate diet was offered twice daily. Considerationa basal diet16,dietary ingredient (Table 1) and chemical compositions in table 2 .

Samples collection and laboratory analysis.

Diet and feed refusals measured and samples were collected daily for five sequential days on the end week. Also the fecal samples were collected twice daily from each goat before each feeding, daily feed consumed was calculated for each goat for digestibility, subsampled, and then stored at −20°C until analysis. Fecal samples were composited for each pen for 5days and subsequently stored at −20°C until analysis. Blood samples, 5 ml from each animal into two tubes with and without EDTA, were collected 2 hours after morning feeding at 8:00 on the final week by jugular venipuncture for CBC analyses and for serum separation by centrifuged for the 10-15 min at 2500 rpm. Sera was separated and stored in refrigerator at −20°C until analyzes for total protein, urea, triglycerides and cholesterolwere determined by using commercial kits,and according to the manufacturer’s instructions. All data received were statistically analyzed using SPSS17.0.

Table 1. The ingredients composition of experimental diet %

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal diet for G1 control group</th>
<th>Treated diet for G2 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Corn</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>Soybean</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Sun-dried Mulberry leaves</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Premix2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Metabolic EnergyMG/kg=CP×0.012 +EE×0.031+CF×0.005+NFE×0.01419.
Table 2. Chemical compositions (%) of experimental feed (DM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dry matter</th>
<th>Organic matter</th>
<th>Ash</th>
<th>Crude protein (CP)</th>
<th>Crude fibers (CF)</th>
<th>Ether extract (EE)</th>
<th>Nitrogen free extract (NFE)</th>
<th>Metabolic energy (MJ/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1diet (control)</td>
<td>89.78</td>
<td>81.86</td>
<td>7.91</td>
<td>15.87</td>
<td>17.21</td>
<td>4.25</td>
<td>62.37</td>
<td>12.41</td>
</tr>
<tr>
<td>G2diet (treated)</td>
<td>87.95</td>
<td>80.20</td>
<td>7.78</td>
<td>15.67</td>
<td>16.99</td>
<td>4.14</td>
<td>60.94</td>
<td>12.15</td>
</tr>
<tr>
<td>Mulberry leave</td>
<td>89.15</td>
<td>83.30</td>
<td>11.75</td>
<td>19.25</td>
<td>13.13</td>
<td>5.50</td>
<td>29.00</td>
<td>7.64</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>88.75</td>
<td>80.86</td>
<td>7.89</td>
<td>14.34</td>
<td>30.46</td>
<td>1.49</td>
<td>46.82</td>
<td>10.25</td>
</tr>
</tbody>
</table>

2 Added per kilogram of dietary DM: 15 mg of Cu, 65 mg of Zn, 28 mg of Mn, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6,000 IU of vitamin A, 600 IU of vitamin D, and 47 IU of vitamin E.

Results and Discussion

Effects of Mulberry leaves on body weight gain and nutrient digestibility.

The bodyweight gain was affected by Mulberry leaves powder in the concentrate diet as presented in Table 3. as shown, goats fed concentrate diet content Mulberry leaves powder, a noticeable increase in body weight while the total gain (kg) elevate (P<0.05) than those goats fed the concentrate diet without content Mulberry leaves. The positive effects of Mulberry leaves in general has been reported by many researchers.

This may be caused improvement in the rumen environment and increased rumen microorganisms, which has a role in raising levels of dry matter digestion and more nutrient absorption. Similar attribution was derived by which showed that the effects of adding flavonoids of Morus leaves, 0 and 2 g/animal/day to the diets of Dorper x Thin tailed ewes has caused an increase in the digestion of organic matter, total nitrogen and fiber extract. Moreover, the flavonoids are available to the animal when adding the berry leaves to the diets lead to improves the overall animals performance, improves digestion, absorption, growth and development of the mammary glands in addition to improving the immune function of the body.

While explained that adding dry berries leaves (Morus) by 0, 0.5, 1.5 and 2.5% of the live weight to the grass hay which provided freely to the Wollo sheep has resulted in overweight at end of experiment at a rate 21.4, 23.2, 25.6 and 25.0 kg/animal respectively. On the contrary, showed no significant differences in body weight for Abergelle sheep supplied to wheat bran and Guizotia abyssinica seeds in addition to the natural grass when adding dry berries leaves 0.25, 50 and 75%. Regarding the nutrient digestibility, the statistical analysis as shown in table 4) revealed that dry matter (DM), organic matter (OM), and other nutrient digestibility, were significantly affected by dietary Morus. This may attribute to the phytonutrients in the Morus leave ‘, impacted diet taste. Furthermore, the presence of flavonoids in leaves enhances the digestive enzyme that has a positive effect on nutrients digestibility. This confirms with mentioned, the flavonoids have been used as feed additives to improve the production efficiency and health of adult cattle. Also consistent with showed that the effect of adding flavonoids of berries leaves, 0 and 2 g / animal/day to the diets of Dorper x Thin tailed ewes has caused an increase in the digestion coefficient of dry matter, organic matter, total nitrogen and fiber extract. Rodriguez mentioned that adding four different of dry tree leaves varieties, including Moringastenopetala), Murule leaves, Trichanthera and Leucaena, by 0 and 1 g to the rumen liquor dairy cattle with fistula which nutrients on natural grass in a free form, the addition led to an increase in laboratory digestion for dry matter and organic matter. While the feeding local male goats on corn bran and green raspberry leaves or dry raspberry leaves have caused an increase in the digestibility of the dry matter.
Table 3. The effects of adding *Mulberry* leaves on body weight gain of female goat (mean ± SE)

<table>
<thead>
<tr>
<th>Parameters groups</th>
<th>initial body weight (Kg)</th>
<th>final body weight (Kg)</th>
<th>Total gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 control</td>
<td>25.30±0.65</td>
<td>27.25±0.45</td>
<td>1,950±0.34B</td>
</tr>
<tr>
<td>G2 treated</td>
<td>25.50±0.78</td>
<td>29.00±0.26</td>
<td>3,500±0.31A</td>
</tr>
</tbody>
</table>

Means with different capital letters in the same Column denoted significant differences at level (P<0.05)

Table 4. The effect of adding *Mulberry* leaves on digestibility parameters of female goat (mean ± SE)

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Digestibility %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry matter (DM)</td>
</tr>
<tr>
<td>G1 control</td>
<td>58.36±0.29 B</td>
</tr>
<tr>
<td>G2 treated</td>
<td>67.83±0.34 A</td>
</tr>
</tbody>
</table>

Means with different capital letters in the same Column denoted significant differences at level (P<0.05)

The effect of adding *Mulberry* leaves on blood biochemical parameters.

The blood parameters investigated the biochemical changes, that may occurs during the nutritional circumstances mainly due to metabolic processes in addition to the correlation with characteristics of ruminal fermentation. Therefore, the changes in some blood parameters may possibly help to explain the beneficial effect of additives in the diet. On this basis, the variations in ruminal NH3-N concentrations and blood urea concentrations were greatly influenced by the feeding patterns of the diet. The effects of *Mulberry* leaves on the blood biochemical parameters are shown in Table 5.

There were differences (P<0.05) in the total protein and blood plasma urea nitrogen. Blood urea nitrogen is the end product of proteolysis of protein metabolism; its concentration is dependent on the crude protein level. Additionally, blood urea nitrogen is negatively correlated with body nitrogen deposition and the utilization rate of proteins. increase blood urea-N concentrations, when increased levels of casein in the diet conception.

These significant (P<0.05) changes it may be dietary *Mulberry* leaves which have positive effect on the nutrient utilization leads to increased blood protein and urea. The similar result concerning total protein and urea were also, reported by via increased digestibility of organic matter and total nitrogen. Whereasa supplemental energy or glucose decreasing the blood urea nitrogen concentrations. The *Mulberry* leaves have improve the nutrients digestion and rumen fermentation by improving the ruminal environment. Regarding triglycerides and cholesterol concentration, they were observed that group fed *Mulberry* leaves with concentrate diet resulted in a significantly less than control group, that agreement with several studies showed that use of *Mulberry* leaves in animals diet, decreased level of serum triglycerides and cholesterol. It is noting that elevated cholesterol can be indicative of dietary lipid content or tissue...
catabolism. On the other hand, that triglycerides and cholesterol was not affected by the supplemented feeds with the Mulberry leaves according to reported by.

Table 5. The effect of adding Mulberry leaves on some blood biochemical parameters of female goat (mean ± SE)

<table>
<thead>
<tr>
<th>parameters group</th>
<th>Total protein gr/100 ml</th>
<th>Urea mg/dl</th>
<th>Triglyceride mg/dl</th>
<th>Cholesterol mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 control</td>
<td>4.15±0.34 B</td>
<td>36.77±0.33 B</td>
<td>22.61±1.25 B</td>
<td>150.93±2.30 B</td>
</tr>
<tr>
<td>G2 treated</td>
<td>6.50±0.31 A</td>
<td>48.67±2.19 A</td>
<td>20.53±0.79 A</td>
<td>147.10±3.77 A</td>
</tr>
</tbody>
</table>

Means with different capital letters in the same Column denoted significant differences at level (P<0.05)

Effect of adding Mulberry leaves on blood parameters.

Statistical analysis was conducted on a group fed diet content Mulberry leaves were compared to the control group, during this experimental period to determine the changes in hematological parameters at the end of the treatment period Table 6. There were a significant differences (P<0.05) in hemoglobin percentage and the mean of PCV%, while the good effect on red blood cell count, white blood cell count, lymphocytes and neutrophils respectively.

The improvement in the hematological parameters in the treated group that is possibility due to an increase in nutrients absorption in the intestine and an increase the microbial activity in the rumen, provide high nitrogenous and condensed tannins, because the presence of condensed tannins could be useful in enhancing rumen fermentation. Also the increasing bodyweight might be enhance the blood-producing to maintenance tissue requirement. In the same direction, the indicated that hemoglobin concentration was significantly (p<0.05) higher in treated group than the control group in goat kids. In addition to the mulberry leaves is rich in nutrients, also contain bioactive substances, such as anthocyanin, flavonoids, jasmonic acid, stilbene, and terpenoids. These bioactive substances have positive effects, such as antibacterial, antipyretic, anticancer, anti-oxidation, hypoglycemic and metabolism-improving properties.

Also, they could be influence the physiology of animals, that confirmed by which reported the Mulberry leaves can be considered safe, and no adverse effects on general behaviour, BW, hematology, and coagulation parameters obtained in the SD rats tested.

Table 6. The effect of adding Mulberry leaves on blood parameters of female goat (mean ± SE)

<table>
<thead>
<tr>
<th>parameters groups</th>
<th>Hb (g/dL)</th>
<th>PCV %</th>
<th>RBCs (106/L)</th>
<th>WBCs (103/L)</th>
<th>Lymphocyte (%)</th>
<th>Neutrophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1(control)</td>
<td>9.62±0.35B</td>
<td>28.87±0.32B</td>
<td>10.06±0.48</td>
<td>9.87±2.8</td>
<td>36.40±1.70</td>
<td>53.50±1.27</td>
</tr>
<tr>
<td>G2 treated</td>
<td>11.90±0.29A</td>
<td>33.13±0.52A</td>
<td>11.47±0.59</td>
<td>10.81±5.8</td>
<td>37.53±0.71</td>
<td>54.80±0.80</td>
</tr>
</tbody>
</table>

Means with different capital letters in the same Column denoted significant differences at level (P<0.05)
Conclusion

This study has compared the female goats' performance variables, between those fed on a standard ration and those that included these ration with Mulberry, that led to positive affects on general performance, blood pictures and biochemical parameters. According to this study, the use of dry Mulberry leaves (2%) from concentrate diet, improve the animal health.

Conflict of Interest: Nil

Source of Funding: Self-funding

Ethical Clearance: Taken from the Scientific Committee, College of Veterinary Medicine, University of Baghdad.

References

18. ALmusawi, J. E. Q. Effect of different levels of whole date on productive performance and some physiological traits in Awassi sheep. Dissertation College of Veterinary Medicine at the University of Baghdad.2013.


In Vitro Production of Ovine Embryo in Non-Breeding Season

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Abstract

This study was undertaken to show the effect of follicular size and culture media on the results of IVF and IVEP in sheep. A hundred ovaries of slaughtered ewes were taken during non-breeding season. The total number of follicles were 256, while the number of recovered oocytes were 189. The recovery rate of oocyte from large and small follicles were 85.8% and 64.7% respectively. Only grades A and B oocyte have been used with a total number of 148 oocytes. Maturation and fertilization rates in different media; MEM, RPMI1640, DMEM low glucose and DMEM high glucose were 64.4%, 51.2%, 32.4% and 26.6% for maturation rate and 66.6%, 55%, 50% and 37.5% for fertilization rate respectively. There was a statistical difference (p≤0.05) in maturation and fertilization rate between different media; the total fertilization and cleavage rate was 56.7% and 70% respectively. The numbers of morula and blastocyst stage were 40% and 35% respectively. There was a statistical difference (p≤0.05) in morula and blastocyst production in different media. It was concluded that abattoir was a good source of oocyte recovery, and the large follicle give the best morula and blastocyst stage. MEM was a good diluent of semen. mMEM give the best result as cultured media as compared with the other media used in this study.

Keywords: IVF, IVEP, Local Iraqi breed sheep Non-breeding season.

Introduction

In Vitro Fertilization (IVF) technique provides large scale, low cost production of early and late stage embryos for gene integration and cloning, expected to make efficient utilization of high numbers of ova left in the ovaries. Ruminant embryo have been cultured in a numbers of defined, semi defined or undefined media. A defined medium should prepared prior to embryo culture by using Identifiable ingredients that facilitate embryo metabolism requirements and promoting culture environment to be similar as it in vivo. IVF procedure includes; Maturation, fertilization of oocyte and culture of zygote until production of blastocyst within 7 days which transferred to recipient or preserved in a liquid nitrogen for future use. There are many factors plays a role in successful of IVF technology including culture media, breeding season, size of follicles, age of recipient, semen donor and the method of oocyte collection. The study was undertaken to establish a reliable procedure of ovine embryo production and effects of certain factors such as season, method of collection of oocytes, follicular size and culture media on embryo production.

Materials and Methods

Oocytes Collections:

Fifty ovine female genital system were collected from abattoir of Fallujah during the period from November, 2019 to the February, 2020, and transported within an hour in a cool box containing NaCl 90% at 33-35°C to the Reproductive Biotechnology Lab., Dept. of surgery and obstetrics, Coll. Vet. Med, Uni. of Fallujah. The ovaries were isolated and subjected to three washing processes with collecting media (MEM, RRMI 1640, DMEM low glucose or DMEM high glucose). Follicles were calculated whether in right or left ovary and measurement of its diameter with an automatic vernier. (2-8mm) follicles were aspirated with a needle (18 gauge) connected with a disposable syringe filled with 3ml of collecting media. The culture media with recovered ova were culture in a well out of 24-wells plate.
Grading of oocytes:

The collected oocytes were evaluated with light inverted microscope according to the classification of 7 grades A, B and C as good, fair and poor respectively in the basis of cytoplasm uniformity and presence of cumulus cells.

The maturation of oocytes in vitro:

Grade A and B ova were chosen and washed two times with maturation medium MEM or DMEM low glucose. Then incubated 38°C, 5% CO₂ with a full humidify environment for 24-27 hours and the incubated wells were evaluated under light inverted microscope. First polar body presence was an indicative of ova maturation 3. Calculation of matured ova were done.

Semen preparation:

Fresh semen were collected from two rams via electro ejaculator (India Instrument, 620 Lesher place, Lansing, MI 48912., USA) and transferred within few minutes to the Reproductive Biotechnology Lab. Semen were evaluated with light microscope then warmed at 35°C, then diluted 1:20 with MEM solution. Heparin sodium (Sundent, China) 10 μg/ml were added to diluted semen for sperm capacitation 3.

In vitro fertilization (IVF):

Diluted capacitated sperm in a value of 1×10⁶ sperm/ml were added to fertilization medium containing mature oocyte kept in a group of 5-8 oocytes for each well, and put it into CO₂ incubator at 38°C, 5% CO₂ and full humidify environment for 24-27 hours 1. The presence of 2nd polar body in the oocyte or sperm head in its cytoplasm was an indicative of IVF within 24 hours of insemination 3. Calculation of fertilized oocytes were done.

In vitro culture:

Zygotes culture were performed in different modified “m” cultural media (mMEM, mRPMI 1640, mDMEM low glucose or mDMEM high glucose) incubated at 38-38.5°C, 5% CO₂ and humidify environment. The development of embryo was noticed every 24 hours and culture media were refreshed by changing 50% of it with a fresh one. 2 to 4 stage proportions were counted within 48 hours. Morula and blastocyst were checked within 120 and 168 hours after fertilization respectively 3.

Analysis of data:

Data were analyzed according to 8 with application of chi-square test & t-test.

Results and Discussion

Follicles numbers counted in 100 ovaries was 256 with a mean of 2.56 follicles for every ovary. The numbers of follicles found in the right ovary was 104 with a mean of 2.08 follicle per ovary. While that found in the left ovary was 152 with a mean of 3.04 follicles per ovary (Table -1). The result showed statistical difference (p≤0.05) in the site of ovary between the right and left one in the numbers of follicles found. Similar observations have been reported by several authors 9, 11. This result might be due to the fact that the left ovary in ewe more active than the right one 12.

<table>
<thead>
<tr>
<th>Type of ovary</th>
<th>Percent of follicles/ovary</th>
<th>No. of follicles</th>
<th>Percent of follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right ovary (50)</td>
<td>2.08±0.2</td>
<td>104</td>
<td>40.6%a</td>
</tr>
<tr>
<td>Left ovary (50)</td>
<td>3.04±0.4</td>
<td>152</td>
<td>59.4%b</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>256</td>
<td></td>
</tr>
</tbody>
</table>

Values: mean ± SE.
Different small manuscripts indicate statistical difference (p≤0.05).
Table-2 explain the effect of follicle size on ova obtained and their grade. The total numbers of small follicles (2-4mm) observed was 142 (55.4%) with a mean size 3.9±0.6. While the numbers of large follicles (5-8 mm) observed was 114 (44.5%) with a mean size of 6.6±0.8. There was a statistical difference in the size of the follicles (p≤0.05) between small and large one. Similar results have been reported by several investigators 1, 3, 11, 13-17. It has been noted that follicular size is affected by several factors; reproductive status of the animals, breeding seasons, age, hormonal stimulation and nutritional state of the animals 10. The results showed that a higher recovery rates were obtained with fair oocyte (grade B) 48.1% (91/189) followed by good oocytes (grade A) 30% (57/189) and poor oocytes (grade c) 21.7% (41/189). There was a statistical difference (p≤0.05) were observed between different grades of oocytes. Similar observations have been made by 7, 18 in ewes, and 19 in goats. It has been reported that high quality oocytes recovered by aspiration in sheep and goats 20. The low quality oocytes obtained might be due to slaughtering of bad quality ewes.

<table>
<thead>
<tr>
<th>Follicle size</th>
<th>No. of follicles</th>
<th>Size of follicle</th>
<th>No. of oocyte recovered</th>
<th>Recovery rate</th>
<th>Grade of oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Small follicle 2-4mm</td>
<td>142a (55.4%)</td>
<td>3.9±0.6a</td>
<td>92</td>
<td>64.7%b</td>
<td>18b (19.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42b (45.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32b (34.7%)</td>
</tr>
<tr>
<td>Large follicle 5-8mm</td>
<td>114b (44.5%)</td>
<td>6.6±0.8b</td>
<td>97</td>
<td>85.8%a</td>
<td>39a (40.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49a (50.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9a (9.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>189</td>
<td>73.8%</td>
<td>57B (30.1%)</td>
<td>91A (48.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41C (21.7%)</td>
</tr>
</tbody>
</table>

Values: mean ± SE.
Different small manuscripts indicate statistical difference (p≤0.05)
Different capital manuscripts indicate statistical difference between different grades (p≤0.05).

In vitro maturation (IVM):

The development of embryo affected the event occurred during maturation of the ova and for the success of IVM, the ova must undergo cytoplasmic and nuclear maturation. Only grade A and B oocytes (148/256) 57.8% of the recovered oocytes were cultured (Table-3). The maturation rate was 45.2% (67/148). Similar recorded has been observed by other workers 3, 5, 18.

<table>
<thead>
<tr>
<th>Follicle size</th>
<th>No. of oocyte</th>
<th>No. of mature oocyte</th>
<th>Maturation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small follicle</td>
<td>60</td>
<td>21</td>
<td>35.0%b</td>
</tr>
<tr>
<td>Large follicle</td>
<td>88</td>
<td>46</td>
<td>52.2%a</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>67</td>
<td>45.2%</td>
</tr>
</tbody>
</table>

Values: mean ± SE.
Different small manuscripts indicate statistical difference (p≤0.05).
The maturation rate of oocyte obtained from small follicles was 35.0% (21/60), while it was 52.2% (46/88) from the large follicle. It was observed that there was a statistical difference (p≤0.05) in the maturation rate between oocytes obtained from large follicles as compared with small one. It has been reported that oocytes recovered from large diameter follicle showed successful development 3, 11, 18, 21 (Fig-1) showed mature follicle with 1st polar body. The effect of culture Media on Maturation rate showed in Table-4, the total Maturation rate in different media was 45.5% (67/148). Highest maturation rate was observed in MEM media 64.4% (27/42), followed by RPMI 1640 51.2% (20/39), then in DMEM Low glucose media 32.4% (12/37) and in DMEM high glucose media 26.6% (8/30). Similar results have been made by several investigators 5, 22, 23. It has been also noticed that the medium used for maturation and its supplemented components such as hormones, and amino acids play a great roles in IVM, IVF and in vitro culture 5, 24, 25. There was a statistical differences (p≤0.05) were observed in a rate of maturation between different media.

<table>
<thead>
<tr>
<th>Type of media</th>
<th>No. of oocyte</th>
<th>No. of mature oocyte</th>
<th>Maturation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM</td>
<td>42</td>
<td>27</td>
<td>64.42%a</td>
</tr>
<tr>
<td>RPMI 1640</td>
<td>39</td>
<td>20</td>
<td>51.28%b</td>
</tr>
<tr>
<td>DMEM low glucose</td>
<td>37</td>
<td>12</td>
<td>32.43%c</td>
</tr>
<tr>
<td>DMEM high glucose</td>
<td>30</td>
<td>8</td>
<td>26.66%d</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>67</td>
<td>45.52%</td>
</tr>
</tbody>
</table>

Media enriched with 10% FBS as a complete medium. Different small manuscripts indicate statistical difference (p≤0.05).

In vitro Fertilization:

<table>
<thead>
<tr>
<th>Type of media</th>
<th>No. of mature oocyte</th>
<th>No. of zygote</th>
<th>Fertilization rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM</td>
<td>27</td>
<td>18</td>
<td>66.66%a</td>
</tr>
<tr>
<td>RPMI 1640</td>
<td>20</td>
<td>11</td>
<td>55.0%b</td>
</tr>
<tr>
<td>DMEM low glucose</td>
<td>12</td>
<td>6</td>
<td>50.5%c</td>
</tr>
<tr>
<td>DMEM high glucose</td>
<td>8</td>
<td>3</td>
<td>37.5%d</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>38</td>
<td>56.7%</td>
</tr>
</tbody>
</table>

Media enriched with 10% FBS as a complete medium. Different small manuscripts indicate statistical difference (p≤0.05).
Table -5 showed fertilization rate in different complete fertilization media (media enriched with 10% fetal bovine serum (FBS)): The total fertilization rate was 56.7-% in different culture media. Similar findings have been made by [16, 22, 23, 26]. Fig-2 showed fertilized oocyte with the second polar body. It has been reported that there are several factors plays a role in successful IVF, such as oocyte collection technique, follicle size, season, age, semen preparation with capacitating agent and cultural media. Fertilization rate was 66.66% for oocytes cultured in complete MEM, while those cultured in complete RPMI 1640, complete DMEM low glucose and complete DMEM high glucose were 55%, 50% and 37.5% respectively. There was a statistical difference (p≤0.05) in fertilization rate among different media. These findings might be attributed to the inclusion of FBS in the media that may give the best results. The percent of fertilization were in the acceptable limit in this works and agreed with. Also the difference in fertilization rate in different media might be attributed to the components of the media.

In Vitro Culture and Blastocysts production:

Table -6 showed the results of in vitro Culture of fertilized oocytes (zygotes) in different stages (2, 4, 8-Cells and Morula stage) till Blastocyst production. The proportion of cleaved zygote was 52.6% (20/38) fig-3, 4-cells fig-4 was 70% (14/20), 8-cells fig-5 55% (11/20) and morula was 40% (8/20) fig-6, the blastocysts production was 35% (7/20) (fig-7). These results agreed with several investigators [3, 4, 22, 23]. It has been observed that the expected Blastocysts percent under in vitro fertilization conditions is around 30% to 40%.

Blastocysts production in a complete MEM medium was 22.2% (4/18), while it was 18.1% (2/11) in a complete RPMI 1640 media, 16% (1/6) in a complete DMEM low glucose and Zero in a complete DMEM high glucose. There was a statistical difference (p≤0.05) observed in blastocysts production in various media. These variations might be due to various factors like, age, breed, technique, media, pH and temperature. Also oocyte quality plays as an important factor for embryonic development [29, 30]. The study suggested that MEIM media give a better result than other media used in this work. Also the study was carried out on non-breeding season so the results show a decrease in development of blastocyst production. So this partially might be due to the effect of season on embryo production.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>No. of zygote</th>
<th>2-cells</th>
<th>4-cells</th>
<th>8-cells</th>
<th>Morula</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM</td>
<td>18</td>
<td>10 (55.5%)</td>
<td>7 (38.5%)</td>
<td>6 (33.3%)</td>
<td>5 (27.7%)</td>
<td>4a (22.2%)</td>
</tr>
<tr>
<td>RPMI 1640</td>
<td>11</td>
<td>6 (54.5%)</td>
<td>5 (45.4%)</td>
<td>4 (36.3%)</td>
<td>3 (27.2%)</td>
<td>2b (18.1%)</td>
</tr>
<tr>
<td>DMEM low glucose</td>
<td>6</td>
<td>3 (50.0%)</td>
<td>2 (33.3%)</td>
<td>1 (16.6%)</td>
<td>1 (16.6%)</td>
<td>1c 16.6%</td>
</tr>
<tr>
<td>DMEM high glucose</td>
<td>3</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0d</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>20 (52.6%)</td>
<td>14 (70%)</td>
<td>11 (55%)</td>
<td>8 (40%)</td>
<td>7 (35%)</td>
</tr>
</tbody>
</table>

All media enriched with 10% FBS as a complete medium. Different small manuscripts indicate statistical difference (p≤0.05).
Conclusions

It may be concluded that abattoir was a good source of oocyte recovery, and the large follicle give the best morula and blastocyst stage. MEM was a good diluent of semen. mMEM give the best result as cultured media as compared with the other media used in this study.

Figure-1: (Mature oocyte).
Figure-2: (Fertilized oocyte “zygote”).
Figure-3: (2-cells stage).
Figure-4: (4-cells stage).
Figure-5: (8-cells stage).
Figure-6: (Morula stage).
Figure-7: (Blastocyst stage).
Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearance: taken from the Scientific Committee and Ethical publication in College of Veterinary Medicine, University of Fallujah.

References


Isolation and Identification of Lactic Acid Bacteria from Buffalo’s Raw Milk in Basrah Province by Sequencing the 16S rRNA.

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Abstract

Milk is a nutrient white fluid that comes from mammary glands of mammals. Lactic acid bacteria (LAB) of raw milk are natural microflora and might be starter cultures for milk fermentation. This study aimed to isolate LABs from raw buffalo’s milk and identify them by 16S rRNA sequencing for possible uses in the manufacture of food product. A total of 30 buffalo’s raw milk samples were collected from different areas of Basrah province (Shut-Alarab, Al-Zubair, Al-Qurna, Basrah conter and Abi-Elkhisib through the period extended from October to December 2019 to isolate the LABs using conventional bacteriology test and identify them by 16S rRNA sequencing using Polymerase Chain Reaction assay (DNA-based method). DNA extraction was carried out in duplicate for all raw milk samples. The results showed buffalo’s milk raw showed positive result for bacteriological test (Gram positive-catalase negative) and for the presence of the 16S rRNA gene. There was no significant difference among ages, months, and area of the current study. Buffalo’s raw milk at >4-8 year of age showed a higher ratio of LABs (71.43%) compared with that of buffalo at >1-4 year of age (47.83%). In addition, the highest ratio of LABs in buffalo’s raw milk was in October (66.6%) and the lowest one was in November (36.3%). Furthermore, the highest ratio of LABs in buffalo’s raw milk was in Abi-Elkhisib (100 %) and the lowest one was in AL-Zubair (20%). In conclusion Lactic acid bacteria were found in buffalo’s raw milk. Biochemical and genotypic tests are effectively used to identify the LABs. All LABs isolates could be survived well in buffalo’s raw milk in any age per year, months, and area of study. These findings suggest the possibility that LABs isolates from buffalo’s raw milk might use to inhibit pathogenic and spoilage bacteria in the food products and might improve flavour and quality of the food products.

Key words: Lactic acid bacteria, 16S rRNA gene, buffalo’s raw milk, starter cultures and probiotics

Introduction

Raw milk is a nutrient white fluid that comes from mammary glands of mammals. It is the main source of nutrition for infant. It consists of a wide variety of bacterial species as a natural microflora of animals and human being. The majority of them belong to genera of lactic acid bacteria. A lactic acid bacterium (LABs) is widely distributed in raw milk, dairy products, and decaying plant materials.

A lactic acid bacterium is considered the most general and significant starter cultures used in fermented dairy products. It has been applied in milk fermentation process worldwide. Milk fermentation process has been relied on the activity of LABs which produce organic acid as the end product of carbohydrates fermentation. In addition, LABs are widely used in inhibiting pathogenic and spoilage bacteria in the food products. Furthermore, LABs in milk are used to improve of gastrointestinal disorders and prevent of certain allergies. LABs used as preservation substance and improving both of flavour and quality of the food products. Applications of LABs have a long history in developed countries. Significant importance of LAB in food industry and health enhancement has prompted...
the developing countries to isolate and identify LABs from raw milk animals and optimize them for industrial applications. Recently, many scientists around the world (Iran, Malaysia, Egypt) have been working on LABs. From this point of view, this work aimed to isolate the LABs from raw buffalo’s milk and identify them by 16S rRNA sequencing for possible uses in the manufacture of food product.

**Material and Methods**

**Samples collection**

A total of 30 raw milk samples were collected from different areas of Basrah province (Shut-Alarab, Al-Zubair, Al-Qurna, Basrah center and Abi-Elkhasib). Before sample collection, udders were washed and dried. Then, the milk samples were collected in the sterile tubes, transferred to the laboratory in an ice box, and stored at -20°C until analysis. The samples were collected from five different regions of Basrah province through period extended from October to December 2019.

**Isolation of lactic acid bacteria**

For LABs isolation, raw milk sample (0.1 ml) was spread on the de Man, Rogosa & Sharpe agar surface (MRS agar, Merck, Germany) and the plate was incubated at 37°C for 24 h. A loopful an overnight culture was then transferred from MRS agar, inoculated in the MRS broth (10 ml), and incubated at 37°C for 24h. From each tube, 0.1 ml was cultured two times on the surface of the MRS agar (Merck, Germany) for further purification. The streaks plating were then incubated using anaerobic incubation jar at 37°C for 48h to provide a good environment condition for growing LABs. After incubation, colonies with distinguished morphologies (white and cream colonies) was swabbed on clean slide, stained with Gram’s stain, and examined under light microscope.

**Biochemical test**

For catalase test, a small amount of white and cream colonies was swabbed on clear slide using wooden stick and a drop of H₂O₂ was then added. Gas bubbles evolutions indicate a positive reaction. Colonies with distinguished morphologies (white and cream colonies) and physiologies (Gram positive-catalase negative) were collected. To identify LABs isolates, 16S rRNA gene sequences were analysed by PCR.

**Analysis of the 16S rRNA gene sequences**

**DNA extraction and detection**

DNA extraction was carried out in duplicate for all raw milk samples using the genomic DNA extraction kit (Qiagen- Germany). The DNA extraction kit consists of GB Buffer, W1 Buffer, Wash Buffer, and Elution Buffer. DNA extraction was performed according to the manufacturer’s instruction manual in following steps: Cell harvesting, lysis, DNA binding, washing, and DNA elution. For cell harvesting: bacterial cultured (1 ml) was transferred to a microcentrifuge tube (1.5 ml), centrifuged at 8,000 rpm for 1min to discard the supernatant. Lysozyme buffer (200μl) was added to the tube to re-suspend the cell pellet at room temperature. For lysis: GB buffer (200 μl) was added to the tube in water bath at 60 °C for 10 min to lysate. For DNA binding: absolute ethanol (200 μl) was added to lysate and mixed thoroughly, transferred to the GD column in 2 ml collection tube, centrifuged at 8,000 rpm for 2min. For wash step: W1 buffer (400 μl) was added to the GD column in 2 ml collection tube and centrifuged at 8,000 rpm for 30 sec to dry the column matrix. Then, wash buffer (600 μl) was added to the GD column in 2 ml collection tube and centrifuged at 8,000 rpm for 30 sec to dry the column matrix. For DNA elution: The dried GD column was transferred to microcentrifuge tube (1.5 ml). Pre-heat elution buffer (100 μl ) was then added to the center of the column matrix, stand for at least 3 min to ensure the elution buffer absorbed by the matrix, and centrifuged at 8,000 rpm for 30 sec to elute the purified DNA.

**PCR primers and amplification**

The primers described in previous study (F: 5’-GCGGCGTGCTAATACATGC -3’; R: 5’-ATCTACGATTTCACCCTAC -3’) were used to amplify a 16S rRNA gene sequences (700bp).

**Polymerase Chain Reaction assay for gene amplification**

DNA from the samples was amplified in a total reaction volume (25 μL) containing genomic DNA (1μg), primers (1μM), Mgcl2 (2mM), dNTP (0.2mM), PCR buffer (2.5μL of 10X) and the enzyme Taq DNA polymerase (1unit). The Polymerase Chain Reaction (PCR) assay was done as follows: Initial denaturation step (95°C for 5 min) followed by 30 cycles of: Denaturation step (95°C for 1 min), annealing step (42°C for 1 min),
extension step (72°C for 1 min), and a final elongation
(72°C for 10 min) using a thermocycler(Techne-UK)
(12,13).

**PCR products detection** The amplified PCR product
was detected on agarose gels (1.5%) prepared with
agarose in Tris-borate-EDTA buffer (1x), stained with
a fluorescent stain, and images by gel-documentation
systems. The size of the band was selected by comparison
with a standard (100 bp) DNA ladder 14.

**Statistical analysis** The Pearson’s chi-square test
was done by using statistical program, SPSS 15

**Results and discussion** In the current study, the
LABs was isolated from buffalo’s raw milk, identified
through conventional bacteriology and genotypic tests,
and investigated their distribution rates based on age per
year, months, and area of study.

**Biochemical test**

In the present study, the isolation results of LABs
from buffalo’s raw milk using MRS medium revealed
that 16 out of 30 (53.3%) were positive for Gram stain,
negative for catalase test, and live under anaerobic
condition. This finding is in agreement with previous
study in which that conventional phenotypic method
(biochemical reactions-based method) using MRS
medium is suitable to identify the LABs in the breast
milk and both of cow and goat raw milk providing a
reliable identification of the isolates 13.

**Polymerase Chain Reaction (PCR) assay**

In the current study, 16 out of 30 (53.3%) buffalo’s
raw milk showed positive result for the 16S rRNA
gene. The amplification length of the 16S rRNA gene
was 700bp (Figure 1). Gene sequences (16S rRNA) for
the identification of buffalo’s raw milk was effectively
done by using PCR. This finding is in agreement with
previous study, in which that sequencing of the V1
region (700 bp and 90 bp) for the gene sequences (16S
rRNA) are sufficient to identify the LABs in the breast
milk and both of cow and goat raw milk providing a
reliable identification of the isolates 16. It has been found
that molecular method (DNA–based method) is possibly
accurate for identification of bacteria 17.

![Image](image_url)

**Figure 1**: The amplification of the 16S rRNA gene (700bp) by using PCR in agarose gel electrophoresis of
DNA. M: ladder (100 bp); lane (1-19) raw milk samples.

**Distribution of LABs in buffalo’s raw milk based
on age per year**

The difference among ages showed that there was
no significant difference (P>0.05) of LABs in buffalo’s
raw milk. Raw milk of buffalo at age >4-8 year showed
a higher ratio of LABs (71.43%) compared with that of
buffalo at age >1-4 year (47.83%)(Table1). This finding
is in agreement with previous study, in which ages did
not effect on the LABs in goat’s raw milk (13).However,
this finding disagreement with previous study, in which
ages effect on the LABs in cow’s raw milk. It has been
found that the number of parturition significantly effect
on the LABs distribution in cow’s raw milk 13.
Table 1: Distribution of LABs in buffalo’s raw milk based on age per year

<table>
<thead>
<tr>
<th>Age /year</th>
<th>Examined N.</th>
<th>Positive N.</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1-4</td>
<td>23</td>
<td>11</td>
<td>47.83%</td>
</tr>
<tr>
<td>&gt;4-8</td>
<td>7</td>
<td>5</td>
<td>71.43%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>16</td>
<td>53.33%</td>
</tr>
</tbody>
</table>

Chi-Square(df=1)=1.20; P value =0.27

Distribution of LABs in buffalo’s raw milk based on months study

The difference among months showed that there was no significant difference (P>0.05) of LABs in buffalo’s raw milk. Raw milk of buffalo revealed the highest ratio of LABs was in October (66.6%) and the lowest one was in November (36.3%) (Table 2). This finding disagreement with previous study, in which there is a seasonal variation during the long study period (January till December, 2014) in the microbial composition and quantity of cow’s raw milk compared with the short present study period (October till December)18.

Table 2: Distribution of LABs in buffalo’s raw milk based on months study

<table>
<thead>
<tr>
<th>Months</th>
<th>Examined N.</th>
<th>Positive N.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>9</td>
<td>6</td>
<td>66.6%</td>
</tr>
<tr>
<td>November</td>
<td>11</td>
<td>4</td>
<td>36.3%</td>
</tr>
<tr>
<td>December</td>
<td>10</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>16</td>
<td>53.3%</td>
</tr>
</tbody>
</table>

Chi-Square(df=2)= 2.09; P value =0.35

Distribution of LABs in buffalo’s raw milk based on region of study

The difference among regions, in which buffalo’s raw milk was whereabouts, revealed that there was no significant difference (P>0.05) of LABs in buffalo’s milk. Raw milk of buffalo revealed the highest ratio of LABs was in Abi- Elkhasib (100 %) and the lowest one was in AL-Zubair (20%) (Table 3). This finding is in agreement with previous study, in which the region studies did not effect on the LABs in raw milk 13.
### Table 3: Distribution of LABs in buffalo’s raw milk based on regions of study

<table>
<thead>
<tr>
<th>Region</th>
<th>Examined N.</th>
<th>Positive N.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shut-Alarab</td>
<td>4</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>AL-Zubair</td>
<td>5</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>AL-Qurna</td>
<td>12</td>
<td>7</td>
<td>58.3%</td>
</tr>
<tr>
<td>Basrah center</td>
<td>6</td>
<td>4</td>
<td>66.6%</td>
</tr>
<tr>
<td>Abi- Elkhasib</td>
<td>3</td>
<td>3</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>16</strong></td>
<td><strong>53.3%</strong></td>
</tr>
</tbody>
</table>

Chi-Square(df=4)=6.69; P value = 0.15

### Conclusion

A lactic acid bacterium was found in the buffalo’s raw milk. Biochemical and genotypic tests are effectively applied to identify the LABs. All LABs isolates could survive well in buffalo’s raw milk based on any age per year, months, and area of study. These findings suggest the possibility that LABs isolates from buffalo’s raw milk might use as probiotics (live bacteria that are helpful for human beings) and improve flavour and quality of the food products and might use as preserve agents.

**Acknowledgements:** The authors thank the Veterinary Medicine College, Basrah University for offering support to achieve this research. The authors also thank all staff and technicians of the Department of veterinary Public Health for their kindness and useful advices.

**Conflict of Interest:** Nil

**Source of Funding:** Self-funding

**Ethical Clearance:** Taken from the Scientific Committee University of Basrah.

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6. El-Shenawy, M., Dawoud E., Amin G., Shafei, K.,


Evaluating the Efficacy of Some Disinfectants, Sterilizers and Detergents Against Streptococcus Pyogenes Isolates from Tonsillitis Patient in Kirkuk City

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Abstract

The aim of the study was to evaluate the inhibitory effectiveness of some disinfectants, sterilizers and detergents commonly used, including: Dettol (Sabtol), Bleach, ethanol alcohol, liquid soap, dishwashing liquid, ActiveX and Sterile Gel 85 on bacterial growth Streptococcus pyogenes. The study included collecting (150) swabs from Patients referred to the hospital and from private medical laboratories (age groups were 16-40 years), the swabs were taken by (Transport media) containing the prepared agricultural medium and transported to the laboratory for the purpose of isolation and diagnosis.

The results of the bacterial sensitivity test under study for these disinfectants, sterilizers and detergents according to the type and concentrations of the preparation (50, 25, 12.5)%, and that alcohol ethanol, hypochlorite sodium and sterile gel (Gel 85) have a high effect on the growth of bacteria Streptococcus pyogenes and with all the concentrations prepared, and while detergents Liquid and liquid soap, dishwashing, had less effect on the bacteria under study, according to the prepared concentrations.

Key words: Streptococcus pyogenes, disinfectants, sterilizers, chemical detergent

Introduction

Inflammation of the upper respiratory tract is one of the most common diseases, especially the tonsils, and its treatment depends mainly on taking antibiotics. Hand contamination and respiratory secretions such as cold and mucus are among the most important ways for infection to spread between patients or health care workers and social and economic institutions to a healthy person.

The discovery of antibiotics had a great impact on reducing the rate of bacterial infections, which encouraged the production of these antibiotics industrially, but nowadays we find a large number of antibiotics used in treating diseases and bacterial infections, but the effectiveness of these antibiotics is constantly decreasing with the ability of germs to develop Means of self-defense and resistance to the action of antibiotics in several ways. As this resistance has the ability to transmit from one bacterial genus to another that was previously sensitive to a specific antibiotic, and this resistance spreads in direct proportion to the increase in the randomized use of antibiotics.

Sterilization is defined as the process by which all microorganisms and their forms (germs, their spores, filtrates, fungi and their spores) are killed or removed, while disinfection is the process in which part or not all living organisms are removed or killed and it does not affect the spores, germs vary in their sensitivity to sterilizers and disinfectants, the germs negative for the dye of honor are less sensitive to disinfectants than the germs that are positive for the dye of generosity, perhaps due to the nature of their composition and their possession of the outer coating, which may hinder the action of disinfectants, and Mycobacteria is relatively resistant, while spores are highly resistant, and the random and increasing use has led to the emergence of resistant strains and made the disinfectant lose its effectiveness and a few of them have the ability to kill spores, and from the need to touch the disinfectant to the area to be disinfected, because Most of these disinfectants do not have the ability to penetrate into the materials surrounding the micro-organism, such as (blood, pus, wounds). Chemical disinfectants are also characterized by instability as it sometimes decomposes into other compounds that help the growth of the microorganism, and some microorganisms have the ability to transform.
and change to become resistant to disinfectants.  

Antiseptic term refers to non-toxic chemical sterilizers when used externally to disinfect skin or wounds and do not affect living tissues, as for detergents is an important and inexpensive way to prevent the transmission of infection in microorganisms because they are effective in removing contaminants that include bacteria, viruses or parasites. As Antibacterial or Antimicrobial, which is characterized by containing the compound Ingredients with an active anti-microbial activity, the current industrial cleaners are made either from petrochemicals (derived from fats and oils) or other chemical materials such as (sulfur trioxide, sulfuric acid, ethylene oxide and alkalis) and all have microbial effectiveness. Bactericidal.

Studies indicate that this efficacy increases with an increase in temperature, so washing hands with soap and hot water eliminates a large part of the pathological bacteria, and the vital effectiveness of these compounds may be due to their entry into the cells cytoplasm and its interference in its own cell interactions to form metabolites that lead to bacterial cell death or The similarity of fatty acids to compounds with those that enter into the formation of the cell wall, which leads to the failure to complete its construction and consequently the death of the cell, or the effect may extend to itself, which leads to an increase in its clarification of these cells and consequently their death.

The bacteria *Streptococcus pyogenes* is one of the common causes of disease events in humans and is responsible for at least 616 million cases of tonsillitis and pharyngitis per year in the world and 111 million cases of infections in children in developing countries as well as causing many diseases, including tissue inflammation. Cellular and cutaneous trauma and toxic shock syndrome, *Streptococcus pyogenes* susceptibility to infection is due to its possession of many virulence factors, including capsule, surface proteins of the cell wall.

Given the importance of evaluating the effectiveness of sterilizers, disinfectants and detergents against *Streptococcus pyogenes*, we decided to conduct this study, which aims to measure the effectiveness of disinfectants and sterilizers, in inhibiting or killing bacteria.

**Materials and working Methods**

Collecting (150) swabs from the nasopharynx area from patients (male and female) using ready-made swabs containing the culture medium, after they were transferred to the laboratory, they were incubated for 24 hours, after which they were implanted on a blood agar medium and incubated on 37°C for 24 hours, (20) of the bacteria *Streptococcus pyogenes* were isolated, and was diagnosed based on the dorsal characteristics that included the size, shape, color, texture of the colonies, and the type of hemolysis, and then microscopically diagnosed for the purpose of describing the shape of the cells by staining them with the Cram statin, Catalase test, sensitivity to bacitracin test, APE20.

| Table (1) Types of disinfectants, chemical sterilizers and detergents used: |
|---------------------------------|------------------|---------|
| **Trade Name** | **The scientific name** | **Origin** |
| 1 | Dettol (Sabtol) | Chloroxylenol | Iraq |
| 2 | Bleach | Hypochlorite Sodium | Iraq |
| 3 | Ethanol alcohol | Ethyl alcohol | Iraq |
| 4 | Liquid Soap | Bright | Iraq |
| 5 | Dishwashing liquid | O2 | Iraq |
| 6 | Sterile gel | Activex | Turkey |
| 7 | Sterile gel | Aniosgel 85 NPC | France |
The test to detect contamination of disinfectants before performing an allergy test:

For the purpose of ensuring that the disinfectants used in this study are free from bacterial contamination, in order to conduct a sensitivity test for bacteria, the following test was performed according to the method 2,11

1- Inoculating the blood agar with a spreading method with (0.1) ml of disinfectant and incubating at 37 °C for 7 days.

2 - Take (1) ml of antiseptic and add (1) ml of the (heart and brain broth), the tubes were incubated at 37 °C for 7 days. The appearance of more than 5 colonies on the plate and the appearance of turbidity in tubes in the middle of the infusion of the heart and brain broth (BHI) indicates Contamination of the disinfectant and its unfit for testing.

Preparation of disinfectant concentrations:

According to the method 2, The stock solution was prepared for seven sterilizers and disinfectants (Dettol (sabot), Bleach, liquid soap, sterile gel, dishwashing liquid, ethanol alcohol) by taking 10 ml of commercially prepared concentrations of these chemical disinfectants and disinfectants and adding it to 90 ml of Sterile distilled water, The final concentration becomes 100%, and under sterilization conditions the following concentrations were prepared (12.5, 25, and 50%).

The sensitivity of bacterial isolation testing to disinfectants, sterilizers and detergents using the diffusion drill method:

<table>
<thead>
<tr>
<th>Types</th>
<th>Average inhibition zone diameters measured in (mm) according to the concentrations used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>Dettol (sabot)</td>
<td>R</td>
</tr>
<tr>
<td>Bleach</td>
<td>(30) S</td>
</tr>
<tr>
<td>Ethanol alcohol</td>
<td>30 &gt;S</td>
</tr>
<tr>
<td>Dishwashing liquid</td>
<td>R</td>
</tr>
<tr>
<td>Liquid soap</td>
<td></td>
</tr>
<tr>
<td>Activex sterile gel</td>
<td>R</td>
</tr>
<tr>
<td>Gel 85 sterile gel</td>
<td>30 &gt;S</td>
</tr>
</tbody>
</table>

Results and discussion:

20 samples of Streptococcus pyogenes were isolated out of (150) swabs, and were diagnosed based on Phenotypic, Microscopic, Chemical and APE20 tests.

Sensitivity testing of bacterial isolation to disinfectants, sterilizers, and detergents:
Table (2) shows the bacterial isolation under study showed different sensitivity to the disinfectants, sterilizers and detergents used, and this discrepancy was evident depending on the type and concentration of the disinfectant used. The results showed that all the disinfectants used at a concentration of 100% have a great effect on the growth of bacteria compared to other concentrations, and the disinfectants (Bleach, ethanol alcohol and Gel85) are among the most sterilizers and disinfectants that affect the growth of *Streptococcus pyogenes* and in all the prepared concentrations, as shown in picture (1) This is consistent with the study 5 which indicated that *Streptococcus spp*. Kill with specified time and concentration when exposed to the Bleach, The Bleach is one of the disinfectants with a wide range of effectiveness against many microbes by destroying bacteria cells through the production of hypochlorous acid, which is a strong oxidizing agent that binds directly with cellular membrane proteins and enzymes as well as its effect on DNA by inhibiting its synthesis in the bacterial cell, Likewise, ethyl alcohol has the advantage of its ability to affect bacteria by drawing water from the cell and draining it or depleting it inside the cell, working on coagulation, denaturing protein and depositing fats 15.

As for the antiseptic Dettol, the bacteria under study showed resistance to it at the concentration (12.5), while the sensitivity to it was shown at the concentration (25)% in the inhibition area (25) mm and at the concentration (50)% in the inhibition area (30) mm, as in the picture (2), And Al-Khalidi indicated in her study that Dettol disinfectant was the least effective on its bacterial isolates, and this is not in agreement with the current results that showed that the dettol has a high efficiency in inhibiting the bacterial isolation under study at a concentration of (25 and 50)% 5.

Activex sterile gel had an effect on the growth of the bacteria under study at the concentration (25)% in the inhibition area (16) mm and at the concentration (50)% in the inhibition area (18) mm, while the bacteria did not show any sensitivity at the concentration (12.5%), as shown in Image (2).

As for detergents, liquid soap and dishwashing liquid are among the least disinfectants affecting the growth of bacteria under study, especially at concentrations (12.5 and 25)% compared to a concentration of (50)%, as in picture (3), The present study agrees with Rama when it was mentioned that the detergent inhibitory concentration increases with the increase in the detergent concentrations used in the study, due to a number of components included in the composition of the detergents that work to inhibit the metabolic activities and kill the bacteria 18,4.

The resistance of bacteria under study to sterilizers, disinfectants and chemical cleaners may be due to the bacteria acquiring the characteristic of resistance resulting from mutations that lead to mutations in cellular metabolism or the bacteria acquiring resistance genes from plasmids or transposone genes, or the occurrence of a change in the target sites or a change in the permeability of the outer membranes of the bacterial cell walls 4,2.

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**Image (1) The effect of ethyl alcohol, Gel85 and Bleach on the growth of *Streptococcus pyogenes* and at all the prepared concentrations.**
Conclusions

The results of this study showed that the most disinfectants and sterilizers affected the growth of *Streptococcus pyogenes*, with all prepared concentrations being alcohol Ethanol, Bleach and Sterile gel Gel 85, and while detergents, Liquid soap and Dishwashing liquid, had less effect on the bacteria under study, according to the concentrations used.

I recommend that chemical disinfectants and sterilizers be used randomly and without referring to the manufacturer’s instructions and installed on the packaging of the chemical disinfectant, which makes the disinfectants lose their effectiveness due to inappropriate use and in the wrong place or because they are used in inappropriate concentrations, as this leads to the emergence of many resistant bacterial strains. For many disinfectants, sterilizers and antibiotics alike, which results in a serious problem that lies in the difficulty of controlling infectious diseases, and conducting a molecular and genetic scientific study to find out the relationship between bacterial resistance to antibiotics and their resistance to disinfectants and chemical sterilizers.

Conflict of Interest: Nil

Source of Funding: Self-funding
Ethical Clearance: Taken from the Scientific Committee of Education Directorate of Kirkuk.

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Study the Role of Selenium or Zinc as Organic form on some Antioxidant and Liver Enzymes of Rams

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Abstract

This study was conducted to detect the role of the organic form of Selenium or Zinc that supported with yeast (Saccharomyces cerevisiae) on the liver function and antioxidant enzymes. Twenty-one rams that their average weight of 38±2 kg. The males divided into three groups. The first group (G1) late without treatment, group two (G2) was administrated with (Saccharomyces cerevisiae-Zinc) (0.2 g/kg/ body weight), while group three (G3) was administrated with (Saccharomyces cerevisiae-Selenium) (0.03 g/kg/ body weight). Blood samples were collected from males at zero 45 and 90 days of experiment. The results of AST, ALT and ALP showed non-significant differences between different groups. While the activity antioxidant enzymes recorded a significant improvement, through a significant increased (P≤0.05) of Glutathione peroxidase and catalase concentrations of the group that treated with organic selenium or zinc compare with a control group, from another hand, the treated group with organic selenium has been shown the significant increases (P≤0.05) of Glutathione peroxidase compared with the animals that treated zinc at the third period. Whereas, the treated groups recoded a significant increment (P≤0.05) with the progress of ages. From results concluded that the feeding selenium and zinc as an organic form that fortified by yeast Saccharomyces cerevisiae significantly increases antioxidant enzyme activity and have a protective role to the liver.

Keywords: Selenium, Zinc, Yeast, Antioxidant, Sheep.

Introduction

Animals production depend on a healthy and normal physiological status. The disorder in the oxidative stability and the physiological factors in the body caused many problems in animal and the human body. Alteration in the activity of the ant oxidative enzymes is a considerable bio indicator due to the significant role of ant oxidative enzymes in establishing the reactive oxygen species stability of the organism. Trace elements play an important role in certain physiological and biochemical processes and vitally important Human and animals development. Therefore, it is important to provide trace elements to a ration of the animal, especially ruminant, the imbalance of antioxidants which triggers dramatic changes in the behavior of an animal as well as the influence of livestock production and also some modifying the blood components and causes diseases that may not respond to the medication. Dietary antioxidants such as vitamins have been reported to inhibit physiological disorders, thus increasing the performance and consistency of the meat. Selenium is complicated in cellular antioxidant protection by the action of glutathione peroxidase, a self-dependent enzyme catalyses, its significant decrease of hydrogen peroxide as well as organic peroxide to water and the corresponding stable alcohol, while still suppressing the production of free radicals. Selenium, it is one of the rare and naturally occurring elements in many foods that are used as a nutritional supplement, and it is one of the necessary and important nutrients for organism, and it consists of further than about 20 kinds of selenoprotein, which have important role in the thyroid hormone activity as well as the construction of DNA, in addition antioxidant and protection against many infections as well as in reproduction.

Since there was an acute deficiency of selenium in the blood of sheep, it was found necessary to add selenium to their ration, which is among the necessary requirements for the animal to provide the largest production and health of the animal. zinc, it is the instant and most copious element and it is very necessary for all living organisms, as it does not oxidize under biological situations, and
this describes why the element zinc achieves important physiological roles and is different from biological processes. It cannot be stored in the body so it requires its addition its importance in promoting growth, it is an anti-bacterial agent, and it regulates interactions and reproduction in animals. It also affects the activity and potency of antioxidant enzymes.

The using Saccharomyces Cerevisiae is necessary to enhance the microbial growth and enhancing the permanence of the fermentation of rumen. From another hand, the supplement yeast be responsible for most of the important mineral nutrients during the digestion process that will positively affect the microbial assemblages and their functions in the rumen, as many studies have indicated that there are beneficial effects of the yeast on the number and activity of microbes in the rumen. Therefore, this study aimed to the study the role Saccharomyces cerevisiae fortified with organic selenium and Saccharomyces cerevisiae fortified with organic zinc as an organic form on the enzymes transporting amines (ALT), (AST) and (ALP) and antioxidant enzymes in the blood serum of domestic male sheep as protective and improvement effect.

Materials and Methods

This study was conducted on the Twenty-one males sheep 1 to 1.5 years of age, with an average weight 38±2 kg. The animals were fed with concentrated feed, as recommended (2%) of the body weight of all study animals, while hay and alfalfa were fed ad libitum. The animals were divided into three treated groups of equal number (seven males for each group). The first treatment group (G1) left untreated as a control group. Second treatment group (G2) was administrated the yeast (Saccharomyces cerevisiae) fortified with organic zinc at a dose of 0.2 g / kg. Live weight) according to 13, the third treatment (G3) was administrated yeast (Saccharomyces cerevisiae) fortified with organic selenium at a dose of (0.03 g / kg / live weight) according to 13.

Samples of blood were collected from the jugular vein throughout three time periods. (beginning (Zero time), middle (45 days) and end of the experiment (90 days), which lasted for 90 days, to measure the level of amine transporting enzymes, which are amine transporter alanine (ALT), amine transporter aspartate (AST) and alkaline phosphatase (ALP) in the blood serum by CORMAY Kit made in Poland by an auto biochemistry analyzer (Model accent 200) made in Poland.

Statistical analysis between the parameters was performed for each time period using Analysis of Variance for complete random design, and the Least significant difference between the means of the different parameters used to identify significant differences under P≤0.05 by using the statistical program SPSS. Issue 25.

Results and Discussion

Table (1, and 2) it has been shown non-significant differences in the concentration of AST, ALT and ALP between the experiment treated groups over the length of the study period, but a significant increase (P≤0.05) was observed during the time period within the one column for the three treatments compared to the first week of the experiment. The results of the current study are in agreement with 14, who found, the adding selenium with vitamin E at 2 ml/animal, showed non-significant differences between treated groups, and agreed with 15 when adding zinc as organic and inorganic forms, observed non-significant differences in ALT concentration. As well as the results are agreement with 16, who recorded that were non-significant differences in the enzymes transporting the amine ALT, AST and ALP after added organic and inorganic selenium (sodium selenate) at a concentration of 0.3 mg/kg feed in lambs. Also, the results were agreement with 17 who observed, non-significant differences in the level of ALT, AST and ALP between the treatments compared with the control when administering Saccharomyces cerevisiae yeast. While the results disagree with 15 who found, the adding zinc as organic and inorganic forms, caused an increase in the level of ALP and a decrease in AST, and disagreed with 18 when adding the inorganic selenium and zinc and their mixtures, an increase in the level of ALT and AST. From other hand, results agree with 19,20, whereas recorded increased ALT, AST and ALP in the selenium addition group compared with the control group. The major benefit high intake of Se is to preserve liver damage under certain circumstances 21, 22. Dietary Se has been reported to protect toward toxic substance, leading to reduced serum ALT. While 23 has been recorded a dramatic increase concentration of zinc in the serum, a decline concentration of CRP, II-6 and TNF-a in the ability to respond to zinc supplementation. In the inflammatory condition, adipose tissue generates cytokines including (interleukin-6) IL-6, that the liver release of C reactive protein. Certain researches have also shown beneficial properties of zinc toward oxidative
damage 24.

Table (3) showed that there are significant differences (P≤0.05) in the concentration of antioxidant enzymes between different treated groups during the study period. The results recorded a significant increment (P≤0.05) of Glutathione peroxidase and catalase concentration of the group that treated with Saccharomyces-selenium or Saccharomyces-zinc compare with a control group, from other hands, the animals that treated with Saccharomyces-selenium showed a significant increment (P≤0.05) of Glutathione peroxidase compared with the animals that treated Saccharomyces-zinc at the third period. Whereas, the treated groups recoded a significant increment (P≤0.05) with the progress of ages. The results of the study are agreed with 25, 26 who concluded the zinc and selenium have improved effect on antioxidant enzymes in chicken muscles, results are also consistent with results of study 27, 28 that the glutathione peroxidase in the meat of beef and hogs meat was increased when they had been fed with saccharomyces cerevisiae that supported with selenium. This results can explain by the role of zinc and selenium as antioxidants the decreased the against reactive oxygen species and it’s the most major elements of the antioxidant protective mechanism 1. Selenium have a high efficiency of the Se-dependent enzyme Glutathione 29 Selenium plays a lot of significant biological activity, such as the regulation of antioxidant enzymes activity improvement of health and productivity 30.

In ruminants, selenium as an organic form, including selenomethionine, is mainly excellently absorbed compared to inorganic selenium, mainly requires the addition of inorganic selenium to amino acids by microflora 31.

Organic selenium has been much more efficiently absorbed into the body than inorganic selenium, contributing to higher glutathione peroxidase activity in the cow 32. Therefore, using the organic form in the current study. In addition, Zinc helps to reduce oxidative damage by engaging in the production of antioxidative enzymes 33. Biological zinc accumulation prevents the development of ROS products, includes, superoxide anion and radical hydroxyl and H2O2 34. Zinc play a pro-antioxidant role and protect against oxidative stress 35. Zinc consumption improves the scavenging functions including its antioxidative enzymes Catalase and glutathione toward oxidation 36 these roles and function of Se and Zn have act a protect the livers and other organs.

Table 1. Showed AST and ALT (U/l) concentration in the serum of male sheep.

<table>
<thead>
<tr>
<th>Date Treated</th>
<th>1st period Zero time</th>
<th>2nd period Mid time</th>
<th>3rd period End time</th>
<th>1st period Zero time</th>
<th>2nd period Mid time</th>
<th>3rd period End time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (T1)</td>
<td>59.83±1.28 b</td>
<td>70.91±0.39 a</td>
<td>74.25±0.41 a</td>
<td>6.35±0.25 b</td>
<td>8.55±0.52 ab</td>
<td>12.72±0.83 a</td>
</tr>
<tr>
<td>Zinc+ yeast (T2)</td>
<td>59.71±0.77 b</td>
<td>72.27±0.26 a</td>
<td>71.34±1.17 a</td>
<td>6.98±0.54 b</td>
<td>7.71±0.32 b</td>
<td>12.42±0.41 a</td>
</tr>
<tr>
<td>Selenium + yeast (T3)</td>
<td>57.49±1.22 b</td>
<td>71.77±0.36 a</td>
<td>69.71±0.73 a</td>
<td>6.11±0.62 b</td>
<td>8.69±0.26 b</td>
<td>11.31±0.57 a</td>
</tr>
<tr>
<td>LSD</td>
<td>4.80</td>
<td></td>
<td></td>
<td>2.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The lower case characters indicate significant variations between the times (P≤0.05)
Table 2. Showed ALP (U/l) concentration in the serum of male sheep.

<table>
<thead>
<tr>
<th>Date Treated</th>
<th>1st period Zero time</th>
<th>2nd period Mid time</th>
<th>3rd period End time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (T1)</td>
<td>70.97±1.60 b</td>
<td>75.11±1.63 ab</td>
<td>76.52±1.29 a</td>
</tr>
<tr>
<td>Zinc+ yeast (T2)</td>
<td>70.28±2.65 b</td>
<td>76.67±2.59 ab</td>
<td>79.42±2.35 a</td>
</tr>
<tr>
<td>Selenium + yeast (T3)</td>
<td>71.84±2.52 b</td>
<td>78.78±2.24 a</td>
<td>82.46±2.30 a</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
<td>6.02</td>
</tr>
</tbody>
</table>

The lower case characters indicate significant variations between the times (P≤0.05)

Table 3. Showed Glutathione peroxidase (U/l) and Catalase (KU/l) concentration in the serum of male sheep.

<table>
<thead>
<tr>
<th>Date Treated</th>
<th>Glutathione peroxidase (U/l)</th>
<th>Catalase (KU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st period Zero time</td>
<td>2nd period Mid time</td>
</tr>
<tr>
<td>Control (T1)</td>
<td>31.8 ±6.5</td>
<td>33.2±8.6 B</td>
</tr>
<tr>
<td>Zinc+ yeast (T2)</td>
<td>33.6±4.9 b</td>
<td>37.6±5.1 a AB</td>
</tr>
<tr>
<td>Selenium + yeast (T3)</td>
<td>32.7±6.8 b</td>
<td>38.1±6.2 a A</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>2.6</td>
</tr>
</tbody>
</table>

The lower case characters indicate significant variations between the times (P≤0.05)

The upper case characters indicate significant variations between the treated groups (P≤0.05)
Conclusion

Selenium and zinc as organic form that fortified by yeast Saccharomyces cerevisiae significantly increases antioxidant enzyme activity and have a protective role to liver. Therefore, the dietary organic Se and zinc may exert a favorable effect on antioxidant ability through enhancing enzymes activities.

Conflict of Interest: Nil

Source of Funding: Self-funding

Ethical Clearance: taken from the Scientific Committee and Ethical publication in College of Veterinary Medicine, University of Fallujah.

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Cytotoxic activity of Green Zinc Selenide Nanoparticles Against Hep-G2 Cell Lines

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Abstract

In the current study a new technique between the plasma physics, nanoparticle and Biotechnology, structured (ZnSe) prepared by cold plasma technique using atmospheric pressure plasmajet system, ZnSe NPs were prepared from selenium nitrate and zinc metal sheets to reduce selenium toxicity. The structural and optical properties were characterized by X-ray diffraction (XRD) and absorbance measurements of ZnSe green NPs. The liver cancer cell line (hepG2) was exposed to the ZnSe nanoparticles, and The percentage of cytotoxicity was suppressed after 24, and 48 from exposure, induced cytotoxicity is found to be 70.3% in hepG2 after exposed 48h ZnSe green nanoparticle, and the maximum cytotoxicity of the normal cell line (REF) was also examined 42.62% when exposure ZnSe nanoparticles and 16.6% when exposure ZnSe green nanoparticles.

Keywords: Cancer cell, Cytotoxicity, ZnSe green NPs, HepG2, Plasma jet.

Introduction

Many researchers are highly interested in nanoparticles and the diversity of their fields of application such as electronics, photonics, medicine, which often use toxic substances that have the potential to cause environmental toxicity, cellular toxicity, and carcinogenesis. safe and easy to prepare, non-toxic and strong need no preparation or a long time to prepare them needed techniques have the potential to commonly use medical procedures for bacteria and fungi techniques. Composite nanoparticles have the potential to be used in the supply of antimicrobial compounds for use as agricultural pesticides, the mechanisms of the composition of nanoparticles vary between different plant species. Nanoparticles are material groups with unique characteristics due to their greater surface area in contrast to macroparticles¹. The smaller and larger volume ratio of NPs are the main features that make biomedical applications useful, which leads to many new properties such as easy operation, biomolecular conjugation, etc. Nanomedicine is the most significant nanotechnological technology health conditions and new ways of treating other illnesses, such as cancer.

Nanomaterials are nanoscale particles, which have optical properties, catalytic reactions, and chemical stability because of their high surface-to-volume rates. Such features have motivated researchers to find new techniques for nanoparticle synthesis²,³. Physical and chemical techniques spend less time creating nanoparticles, but defense materials that are poisonous and cause environmental toxicity are needed to protect them. The green method with plants is being increased as an active, healthy, non-toxic, and environmentally friendly method⁴. Nanoparticles like iron oxide, zinc oxide and titanium dioxide are selectively cytotoxic to cancer cells and can be used for the treatment of cancer⁵. Zinc Selenide (ZnSe) is also called II-VI semiconductor because it is a light yellow binary stable compound in which Zinc and Selenium form the 2nd and 6th sets of the periodic table. It is an intrinsic semiconductor with a bandgap of approximately 25 °C of 2.70 eV. Because of the quantity impact and the presence of a relatively large proportion of surface atoms, ZnSe is one of the most common and essential glass materials both for use
Non-thermal plasma processing techniques have emerged as promising all-gas phase protocols for synthesizing a wide range of NPs, including semiconductors, metal oxides, ceramics, and more complex core-shell nanostructures among a variety of potential candidates. Therefore, the preparation of ZnSe-NPs was needed with cold plasma and (core-shell) methods to reduce toxicity in an easy, green, economic and environmental manner. There is growing interest in the use of plasma for biomedical applications, especially under the so-called ‘plasmadrug’ to take advantage of the action of low-power, air-pressure plasmas for therapeutic purposes. We have studied the feasibility of the synthesis of ZnSe NPs with an aqueous solution extracted from Milk Thistle seeds. A very mild and solvent-free procedure for obtaining the herbal material was used at a low temperature. We suggest that this bio-safe extract acts as both a reduction and a stabilizing agent. In terms of Cytotoxicity, the synthesized nanoparticles were evaluated as antitumor agent.

### Material and Methods

Aqueous solution extracted from Milk Thistle seeds, metallic zinc, Deionized water, and Selenium nitrate \([\text{SeO(NO}_3\text{)}_2]\) was Purchased from Al-Bashir Company, Baghdad / Iraq. The Liver (Hep-G2) was obtained from Biotechnology Research Center/ Al-Nahrain University/ Iraq, and (REF) cell line was Obtained from Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyyah University, Baghdad, Iraq.

### Preparation the extract of Silybum marianum

The extract contains the metabolites of cells in addition to their components. The components are usually of anti-oxidant properties, i.e. they act as substances with a reducing ability that converts selenium ions into nano selenium and changes colour after exposure to plasma. Impurities were first removed from seeds which were then washed several times with deionized water. Five grams of the seeds were added to 100 ml of deionized water magnetically stirred for 20 minutes at 60 °C. The extract was placed in the centrifuge device tubes, and the material was reduced to the liquid and filtered with filter paper.

### Green synthesis of Zinc Selenide Nanoparticles (ZnSe NPs)

Selenium nitrate \((\text{SeO(NO}_3\text{)}_2)\) was the source of Selenium in all experiments; it was dissolved in deionized water. In a typical reaction procedure, mixture containing 2 mL of Silybum marianum extract was added to 8 mL of 0.4 mM aqueous solutions of selenium nitrate. The plasma is exposed at the gas flow rate 2 L/min and for a time of 3 min, observed the colour change indicating the obtaining of selenium nanoparticles in the green environment-friendly way. A zinc metal sheet changed the electrode of the system under the same conditions of gas flow rate and time. Thus, the zinc metal enveloped the green nano selenium and altered the colour, leading to the obtaining of the zinc selenide compound in the green method.

### Optical and Structural Measurements

A UV wavelength range of 300 to 1100 nm (Shimadzu UV- 1800 spectroscopy) was used with a double- optical spectrometer (PD-303 UV) to detect Surface Plasmon Resonance (SPR) at room temperature for ZnSe NPs. The structural evolution of the synthesized ZnSe NPs was carried out using an X-ray diffractometer [Shimadzu XRD-6000, AS (3k. NOPC)], with a Cu-Kα -radiation wavelength of \(\lambda = 0.15418 \text{ nm}\) operating at 40 kV and 30 mA in a configuration of \(\theta – 2\theta\). The following Debye-Scherrer equation was used:

\[
\frac{0.9\lambda}{C.S} = \beta \cos \theta \quad (1)
\]

where (CS) is the crystalline size, \((\lambda)\) is the wavelength \((1.5406 \text{ Å})\) of the Xray, \((\beta)\)is the degree
of the peak of diffraction, and ($\beta$) is the full width half maximum (FWHM).

**In Vitro Cytotoxicity Assay**

This study was performed on two types of cells, namely, liver cancer cell line (HepG2,) and normal tissue cell line (REF). For tissue culture, microtiter plates of 96 (12×8) well were used. Each well was used to seed 10,000 cancer cells and incubated at 37°C for 24 hours to generate a monolayer which was confirmed by an inverted microscop. The wells were grouped virtually (four wells in each group) to be ready for the following steps:

Three groups were presented with nanoparticles, namely, Se, ZnSe, and ZnSe with extract MT, by diluting the NPS in the culture medium so that their concentrations for each prepared element were (100, 50, 25, 12.5, and 6.25)µg/ml, and the standard control group (control). The plates were re-incubated at 37°C for three-time intervals of 24, 48, and 72hrs. After incubation, the growth medium was decanted off, and crystal violet dye was added (100μL/well) to stain cancer cells. The cells were then incubated for 20 min at 37ºC, followed by washing of plate by water. Then, an assay Analyzer device was used to Analyze the described staining and calculate the percentage of live cancer cells (inhibition rate). The mean value was computed for each group. The calculated mean of inhibition percentages at 24, 48, and 72 hrs was recorded separately for results analysis. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

\[
\text{Inhibition rate} = \frac{A - B}{A} \times 100 \quad \text{(2)}
\]

where A is the optical density of control, and B is the optical density of the samples.

**Statistical Analysis**

It is implemented using a one way (ANOVA) analysis of variance to verify the least significant difference between all treated groups, and (t-test) analysis of variance to verify between control with each treated groups separately using (Graph Pad Prism 6.00) software. The values were presented as the mean ± SEM of triplicate measurements.

**Results and Discussion**

**Ultra Violet Spectroscopic analysis of ZnSe**

UV-Visible absorption spectroscopy was used for the calculation of the band energy. The optical absorbance was measured at room temperature as a function of wavelength (300-1100 nm) for ZnSe NPs with an average thickness of 200 nm, where the absorption spectrum was declined as previously described.

The bandgap energy of films near the absorption edge was determined using the Taucie method through the following formula:

\[
\alpha h\theta = B(h\theta - E_g)^{1/2} 
\]

where $\alpha$ is the linear absorption coefficient, B is a constant, $h\theta$ is the photon energy, and $E_g$ is the bandgap energy for NPs with direct and indirect bandgap energy. It is possible to calculate $E_g$ of the film by plotting $(\alpha h\theta)^{1/2}$ versus $h\theta$ and extrapolating the straight line to the photon energy axis. The value of $E_g$ is higher than the value of ZnSe bulk optical bandgap (2.7 eV) because of the ZnSenanocrystallitesthe quantum confinement. The energy gap is $E_g = 3.61$ eV in ZnSenano this increase in the energy gap in nanoscale solutions may arise from the effect of quantum volume, agree with shown in Figure 1.

**Fig.1.** Energy gap of ZnSe NPs indirect methods ways from the relationship between $(\alpha h\theta)^{1/2}$ as a function with the photon energy.
surface plasmon resonance peak of ZnSe green NPs at 600nm; this characteristic is evident in the visible light area and is responsible for the colour change when the material reaches the nanoscale. This property causes the temperature around the nanoparticle to rise when the light falls on it. Thus, this feature was employed in many applications, including the inhibition of the growth of cancer cells\(^\text{19}\).

The crystalline size of (ZnSe green NPs) indicates that the sizes of samples prepared were below the nanoscale, as appear in the table (1).

<table>
<thead>
<tr>
<th>sample</th>
<th>2θ (deg)</th>
<th>FWHM (deg)</th>
<th>d-value (Å)</th>
<th>size of crystalline (nm)</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnSegreen NPs</td>
<td>26.09</td>
<td>0.38</td>
<td>3.415</td>
<td>21.51372</td>
<td>24.53397</td>
</tr>
<tr>
<td></td>
<td>36.3</td>
<td>0.14</td>
<td>2.66</td>
<td>34.91644</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.45</td>
<td>0.68</td>
<td>2.008</td>
<td>12.69346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.68</td>
<td>0.3</td>
<td>1.94</td>
<td>29.01224</td>
<td></td>
</tr>
</tbody>
</table>

In the same way, the energy gap of ZnSe\(_{\text{green}}\) NPs was calculated and found to be greater than ZnSe NPs, figure 2 showing the energy gap for ZnSe green NPs.

Structure Properties Using X-ray Diffractions

ZnSe green NPs synthesised in cold plasma showed Bragg’s X-ray (XRD) patterns reflecting ZnSe green NPs fcc structure. Fig. 5 illustrates the X-ray diffraction patterns for ZnSe green NPs prepared where a maximum of four peaks is observed, equivalent to 26.09°, 36.3 °, 45.45 °, and 47.68 °, corresponding to the (111), (101), (220), and (102). This applies to the ZnSegreen NPs in line with ZnSe’s regular X-ray diffraction model (JCPDSno. 01-088-2345). The above pattern demonstrates that no other materials can replicate the diffraction pattern of any peak that indicates the purity of the prepared sample and the absence of external contamination that matches findings from other studies\(^\text{21-23}\).

Effects of (Se, ZnSe, and ZnSegreen)NPs on Liver Cancer HEP-G2 cell line

After 24h incubation of HEP-G2 cells, maximum Cytotoxicity was 60. 73% which was achieved in the case of Se with a concentration of 100%, as shown in Figure 3.
Fig. 3. Growth inhibition rate of HEP-G2 (24hrs incubation at 37°C).

48 h incubation of HEP-G2 cells, resulted in maximum Cytotoxicity of 68.97% recorded in the case of Se NPs with a concentration of 100%, as shown in Fig. 4.

Fig. 4. Growth inhibition rate of HEP-G2 (48hrs incubation at 37°C).

Effects of Se, ZnSe, and ZnSe green NPs on REF Normal cell line

In REF, 24h incubation caused a maximum cytotoxicity of 56.6% in the case of Se NPs with a concentration of 100%, as shown in Fig 5.

Fig. 5. Growth inhibition rate of REF (24hrs incubation at 37°C)

48h incubation of REF cells caused maximum cytotoxicity of 53.57% in the case of Se with a concentration of 100%, as shown in Fig. 6.

Fig. 6. Growth inhibition rate of REF (48hrs incubation at 37°C)

The results of the in vitro test of this research confirmed the selective effects of the treatment of nanoparticles on cells, i.e. that cancer cells were inhibited without any harm for normal cells, where MT reduced Cytotoxicity on normal cells while increased the inhibition of cancer cells. Nanotechnology has opened up a new area in cancer care by monitoring the release of the medication and reducing the side effects.

An additional factor is that the high nanoparticles surface-volume ratio helps different functional groups in the plant to get an attachment to a nanoparticle and thus tie the tumour cells together. In addition, the tumour cells serve as an active site for collecting nanoparticles due to the small particle size of nanoparticles (< 100 nm) and the lack of an appropriate tumour lymphatic drainage system.

The mechanisms by which the ZnSe NPs and ZnSe green NPs applied their toxic impacts on hep-G2 may include apoptosis, necrosis and ROS generation. Generally, the release of dissolved Zn2+ ions inside the cells is the real action of the ZnSe N.P.s and ZnSe green N.P.s for cancer cells in which ROS actions increase causing cancer cells death by an apoptosis signaling pathway.

Conclusions

In summary, we have prepared ZnSe green NPs by cold plasma using zinc sheet and selenium nitrate. We
prepared ZnSe NPs using a green method by the milk thistle seed at room temperature. We tuned the optical properties with the desired particle size of ZnSe-NPs to improve the absorption coefficient that affects the efficiency of the solar cell. All the nanoparticles showed an absorption peak due to their quantum confinement effects in their optical spectra. This method has many advantages in terms of the cost, speed, reproducibility, and meeting all criteria for “green” preparation of ZnSe-NPs. Work also focused on predicting the possible cytotoxic effects of biosynthesized (Se, ZnSe, ZnSe green) NPs on human HEP-G2 liver cancer, and normal REF cell lines.

Acknowledgments

The authors gratefully acknowledge for each, University of Baghdad, College of Science, Department of Physics, Plasma Physics Lab, and Dr. Mohammad M. F. Al-Halbosiy (Biotechnology Research Center/Al-Nahrain University/Iraq), for supporting this work.

Source of Funding: Self funded

Conflict of interest- Nil.

Ethical Clearance: Taken from the Scientific Committee University of Baghdad.

References


Cytotoxicity of lipopolysaccharide extracted from *Salmonella enterica serovar Typhimurium* on Breast Cancer Cell Line mcf-7

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Abstract

Lipopolysaccharide was selected to investigate its effect on cell line of breast cancer *in vitro*, therefore lipopolysaccharide was extract from *Salmonella enterica serovar Typhimurium* followed by evaluation of its cytotoxicity. The experiment was divided into three parts. The first part included isolation and identification of *S. enterica serovar Typhimurium* from 302 samples included 201 diarrheic patient samples and 101 food samples of poultry meat and as well as the isolates identification on molecular level through used of three genes (*invA*, STM4497 and *Stn*) genes detected using the technique of PCR, the results shown there were seven bacterial isolates diagnosed as *S. enterica serovar Typhimurium*. The second part of the experiment was extract of LPS from 10.66 g of dry weight of bacteria by hot phenol method. The concentration of LPS in phenolic phase was 435.57 μg/ml while in aqueous phase was 381.76 μg/ml. The LPS molecular weight was estimate which was equivalent to 93325 for phenolic and 71614 Dalton for aqueous phases. The third part of experiment was to study the effect of cytotoxicity in cell line of breast cancer by used different concentrations of lipopolysaccharide. Lipopolysaccharide showed cytotoxicity on the breast cancer cell line mcf-7 in both phenolic and aqueous phases of LPS. The results showed the highest rate of inhibition of breast cancer cells at the concentration of 350 μg/ml by 61.67% in the phenolic phase while the rate of inhibition was 53.33% in the same concentration of the aqueous phase.

Keywords: Lipopolysaccharide, *S. enterica serovar Typhimurium*, MCF-7 cell line, cytotoxicity.

Introduction

Lipopolysaccharides its endotoxins responsible for many of the biological properties of gram negative bacteria particularly (phosphoglycolipid, lipidA) the lipid component¹. These toxins are the most importance among virulence factors for that bacteria and causing the diseases in both animals and human ². There are especially effects for lipopolysaccharide, including lethal toxicity, low blood pressure, fever, and leukocytosis, which has a role in activation of leukocytes and platelets aggregation ³.

The cancer is one of the highest causes of disease and death in the world ⁴. Breast cancer is one of the most common types among women in the world, as it is considered the main cause of the death of women in 15%. Breast cancer accounts for 26% of all cancer cases diagnosed among women worldwide ⁵.

Some bacterial properties have mechanisms that stimulate the immune system to enhance defenses against malignant tumors ⁶. Among the most important of these characteristics is lipopolysaccharide, which is the main component of the outer membrane of the cell wall of the Gram-negative bacteria, that has an assistant effect in stimulating the immune response ⁷.

The LPS compound have cytotoxic effect through its effectiveness of immune cells which are believed to play an important role in the cytotoxicity of gram
negative bacteria through production of biologically active molecules for example: free radicals mediators, prostaglandins, and pro-inflammatory cytokines. So, the LPS compound is not a toxic molecule in itself.

Lipopolysaccharide increases the resistance of bacterial and viral infections by enhancing of B cell response and stimulates interferon production by T lymphocytes. Some researchers have shown that can increased the migration of human esophageal cancer through stimulation of TLR-4 by LPS and it’s can act important role as a catalyst enabled the treatment of gliblastoma multiforme and colorectal cancer.

Because the modicum of available studies in the use of LPS extracted from bacteria in the inhibition and killing of cancer cell line, the study aimed to extract and partially purification the LPS compound from selected isolate of Salmo nella enterica serovar Typhimurium. Cytotoxicity effect of lipopolysaccharide was determined against breast cancer cell line mcf-7.

Materials and Methods

Bacterial isolation:

A total of 302 samples included 201 diarrheic patient samples were collected from of patients at the Fallujah educational hospital and 101 food samples included poultry meat collected from local markets in Fallujah city, and the consulting office at the college of veterinary medicine in university of Fallujah. The specimens were directly inoculated onto MacConkey and Samonella Shigella agar (oxoid) and were incubated at 37°C for 24 hours.

Identification of S. enterica serovar Typhimurium by PCR assay:

Identification of S. enterica serovar Typhimurium was done by using of PCR assay with three primers. The primers were used to amplify the following genes:

1. 617 bp Stn gene
   F-5' TTGTCTCGCTATCACCC 3'
   R-5' ATTCGFAACCCGCTCTGTCC3' 12

   The amplification was done by using 20 μl of reaction mixture with Taq DNA polymerase 1 U/ 20 μl, dNTP mix 250 μM, Primer F 1 μl, Primer R 1μl, MgCl2 1.5μl and Genomic DNA 3 μl. The program amplification of thermal cycler was performed at initial denaturation 94°C for 1min followed by 35 cycle of 94°C for 1 min, 59°C for 1 min, 72°C for 1 min and final extension 72°C for 10 min. Then amplified DNA fragments were examined by utilizing electrophoresis in agarose gel (1.5%). Gels have been stained with ethidium bromide and were photographed by using gel documentation system with UV light.

2. 523 bp of STM4497 gene
   F-5'GGAATCAATGCCCGCCAATG 3'
   R5'CGTGCTTGAATACCGCCTGTC3' 13

   The amplification was carried out using 20 μl reaction mixture containing from Taq DNA polymerase 1 U/ 20 μl, dNTP mix 250 μM, Primer F 1 μl, Primer R 1μl, MgCl2 1.5μl and Genomic DNA 3 μl. Amplification was performed in a programmed thermal cycler were initial denaturation 94°C for 5min followed by 35 cycle of 94°C for 1 min, 68°C for 2min, 72°C for 2min, final extension 72°C for 10 min.

3. 211bp of invA gene.
   F5'ATCAGTGAGCTGGTCTTATCTGAT 3'
   R5'TCTGTTTACCGGGCATACCAT3' 13

   The amplification was carried out using 20 μl reaction mixture containing Taq DNA polymerase 1 U/ 20 μl, dNTP mix 250 μM, Primer F 1 μl, Primer R 01μl, MgCl2 1.5μl and Genomic DNA 3 μl. Amplification was performed in a programmed thermal cycler at initial denaturation 94°C for 5min followed by 35 cycle of 94°C for 1 min, 60°C for 2min, 72°C for 2min and final extension 72°C for 10 min.

Antibiotics susceptibility:

Antimicrobial susceptibility tests using method of by the disc diffusion technique on Muller Hinton agar . The zone diameter of each isolate was compared with National Committee of Clinical Laboratory Standards (NCCLS) 15. Results were recorded as susceptible, intermediate susceptible or resistant, based on the inhibition zone size of each antimicrobial disc used.
Extraction of lipopolysaccharide:

The selected bacterial isolate was cultured under aerobic condition on S.S. and MacConkey agar (Oxoid) and incubated for 24 hours at 37°C. The isolate was harvested using spreader with phosphate buffer saline pH 7 and then washed twice by same buffer. The cells were precipitated using cooling centrifuge at 3000 rpm/min at 4°C for 15 min. The pellet of cells was resuspended in PBS buffer and centrifuged again for 10 min. The cells were dried by cooled acetone (1:10) in ratio 16.

Destruction of bacterial cells:

Depending on the method of 17, destruction of bacterial cells was done by using enzymes as follows: The dried cells were suspended in PBS pH 7 (0.05M EDTA and 0.05 sodium azide) with ratio 1:10. The lysozyme enzyme was added with ratio 0.1 mg / g from weight of bacteria and the suspension was put in the magnetic stirrer in refrigerator for 18 hours, then the suspension was incubated in water bath at 37°C for 20 min and place the suspension in the magnetic stirrer for 3 min. The volume of the strand was then diluted by adding an equal volume of magnesium chloride solution (0.02M), then add DNAase and RNAase enzymes with a final concentration of 1 mg / ml. Finally, the suspension was incubated at 37°C for 10 min and then incubated at 60°C for 10 min.

Extraction of lipopolysaccharide by phenol:

The suspension of bacteria was preheated at 70°C using a water bath and add 90% of phenol solution in equal volume, previously heated at 70°C 18, then the mixture was placed in the magnetic stirrer with a heating unit at 70°C for 15 min. The mixture was put directly in a snow bath to the temperature of 20°C and then centrifuged in capacity of 18000 g and 3000 rpm for 15 min. After the centrifugation, the separation of four phases from top to bottom were observed as follows: aqueous phase, interphase, phenolic phase and sediment. The aqueous and phenolic phases were separated with a Pasteur pipette (both on one side) and then re-extract the remainder by adding three volumes of distilled water and placing the mixture in the magnetic stirrer for 5 min. then the mixture was centrifuged at the same speed above and separate the floating liquid and add to the aqueous phase. Finally, the phases were dialyzed against D.W. for several times and several days to remove phenol’s odor. Partial purification by gel filtration Sephacryl s 200: One hundred and one milliliters of Sephacryl s-200 gel (Pharmacia) were washed with D.W., then were washed by phosphate buffer saline pH 7.2, degassed under vacuum. Subsequently the suspension was poured into a glass column (1.5× 90cm) and allowed the matrix to settle down. The gel was equilibrated with PBS pH 7.2 with flow rate (4ml/7min). LPS sample were added to the column and washed with PBS buffer, the fractions were collected and the absorbance at 280 nm was measured for detecting of contaminating proteins within fractions 19 at 490 nm to estimate the carbohydrate concentration and measuring the absorbance at 260 nm for detecting the nucleic acids 21. Proteins were measured at a wavelength of 595nm whereas the molecular weight of LPS was determined according to standard proteins (Pepsin 34.5KD, GTF from Sterpt. pneumonia 58.2KD, Bovine Serum albumin 67KD, Arginine Deaminase 143.548 KD and Catalase 232 KD) which were added to column and the ratio of Ve/Vₒ was determined to the standard proteins and they were used as molecular weight markers. Blue dextran was also used for the determination of the column void volume (vₒ) and Ve/ Vₒ was measured for LPS of Salmonella typhymurium isolate. The logarithm of the molecular weight of each standard protein was plotted to obtain standard curve.

Determination of molecular weight for Lipopolysaccharide:

Determination the molecular weight of lipopolysaccharide was done by gel filtration chromatography using Sphacryl S – 200. Blue dextran 2000 was used to determine the void volume, which is equal to 43 ml. Molecular weight of LPS was estimated for both phenolic and aqueous phases by using standard protein and drawing the relationship between the logarithm of standard protein molecular weight and the recovery volume/ void volume (Ve/Vₒ).

Cytotoxicity of LPS on cell line of breast cancer mcf-7:

The cytotoxic of lipopolysaccharide on breast cancer cell line (mcf-7) was studied by MTT (3-(4,5-dimethylthiazol-2-yl)23). The cells were plated on 96 wells at 37°C for 24 hrs. at a density of 104 cell per well, then add different concentrations of LPS 0, 100, 150,
250, 350 μg/ml for both phenolic and aqueous phases. After 72 hours at 37°C of incubated, the cells viability was determined. The medium was removed by addition of 28 μg/ml of MTT and the cells were incubated at 37°C for an one hour and a half, then the MTT solution was removed. The remaining crystals in the wells were dissolved by adding 100 mg/ml of DMSO (Dimethle Sulphoxoide) and after that incubated by a shaking incubator for 15 minutes at 37°C. The absorption was determined using the ELISA at a wavelength of 492 nm with three repeats per concentration. The inhibition rate was calculated by the following equation:

\[ \text{Inhibition rate} = \frac{A - B}{A} \times 100 \]

where A= control and B= density of the cells treated with LPS.

**Results and Discussion**

*S. enterica serovar Typhimurium* used in current study was isolated from 302 samples included 201 diarrheic patient samples were collected from patients at the Fallujah educational hospital and 101 food samples included poultry meat collected from local markets in Fallujah city.

The results shown there were 19 (6.29%) isolates of *Salmonella* spp. included 7 (36.84%) isolates diagnosed as *S. enterica serovar Typhimurium* depending on evidences of morphological and biochemical characterizations.

**Antibiotics sensitivity of Salmonella species:**

Antibiotics sensitivity for clinical isolates of *Salmonella* was done against eleven antimicrobial agents. The results showed that all isolates were resistance to Rifampin at 100% followed by the Doxycycline at 94.74% while the average resistance of Cephalaxin, Cefotaxime and Cefixime was 42.1%, 42.1% and 52.63% respectively.

Ciproflaxacin, Aztreonam, Imipenem and Amikacine antibiotics were more effective against *Salmonella* isolates with a sensitivity percentage 94.74%, 94.74%, 100% and 100% respectively (figure 1).

Aslo, the results showed that all *Salmonella* isolates were susceptible to Ciproflaxacin except the isolate S259 which appeared multi-resist against different antibiotics.

**Identification of Salmonella isolates using PCR assay:**

Most ten of *Salmonella* isolates resistance to antibiotics were selected for the diagnosis by PCR assay using of *invA*, *STM4497* and *Stn* genes to detect the strains of *S. enterica serovar Typhimurium*.

An attempt was made to localize the gene responsible for the susceptibility of the *Salmonella* bacteria to tissue invasion.

Accordingly these *invA* gene (211bp) were amplified using specific primers with PCR cycler and optimized specific program. The results showed that all the selected strains contained *invA* gene, the specificity primers were agreement with stated 24, 25.

The results identification of *STM4497* (523bp) gene to detect *S. enterica serovar Typhimurium* showed that seven isolates gave a positive result of the presence. The specificity were reported on earlier study by 25, because isolates gave specific bonds to the gene.

Also the gene *Stn* (617bp) was detected, which is encoded for the production of intestinal toxin of *S. enterica serovar Typhimurium* the results showed that three isolates only possessed this gene, which agree with 26, but differed in proportions from the current study.

Also, the 27 diagnosed *S. enterica serovar Typhimurium* by PCR technique using a *fimC* gene.

According to the previous results, The selected organism which used for the extraction and purification of LPS was the most resistant to antimicrobial agents as well as having the three studied genes.

**Extraction of lipopolysaccharide**

The dry weight of selected *S. enterica serovar Typhimurium* isolate was 10.66 mg as the yield of obtained bacterial growth. The method which used to extracted of LPS by hot phenol was depend on 17. determination the concentration of the lipopolysaccharide was done by using the standard glucose curve. The concentration of LPS in the phenolic phase was estimated at 435.57 μg/ml, while the concentration in the aqueous phase was
estimated at 381.76 μg/ml.

Because the phenolic phase contains a large amount of lipopolysaccharide also due to the structure of the LPS which containing a large amount of N and O-acetylated-6-deoxyhexose in the side chain O which are hydrophobic bonds, some researchers have used that method and therefore accumulate LPS in the phenolic phase. So, The distribution of LPS in both phenolic and aqueous phases depends on the important factor which is hydrophilic bond due to formation of side bond O.

Figure 1: Susceptibility of Salmonella species
Partial purification of lipopolysaccharide:

Partial purification of lipopolysaccharide for both of phenolic and aqueous phases were done by gel filtration chromatography to separate of high molecular weight protein and carbohydrates. The parts were read on the wavelength 600 nm with flow rate was 34 ml/hour and the void volume was equal to 43 ml.

The measuring of protein amount was done by collection of forty-five fractions and assessed for both phenolic and aqueous phases at a wavelength of 289 nm whereas the amount of carbohydrate linked LPS was measured at 490 nm (figures 2 and 3). The results showed that there were two peaks of protein large and small one linked to lipopolysaccharide and difficult to separate whereas phenolic phase there was one peak of carbohydrate. While in aqueous phase there were three peaks of protein: two large and small one linked to the carbohydrate and there was one peak of carbohydrate.

Figure (2): Gel filtration chromatography of *S.*typhimurium (phenolic phase) lipopolysaccharide by using Sphacryl S – 200, the column dimensions was (1.5 x 70 cm) and elution was done with phosphate buffer saline pH 7.2 at flow rate 34 ml/h
Determination of lipopolysaccharide molecular weight:

Results for determinate of lipopolysaccharide molecular weight showed that the molecular weight for LPS in phenolic phase was equal to 93325 Dalton and in aquatic phase was equivalent to 71614 Dalton as shown in figure (4) and table (1). The molecular weight of the lipopolysaccharide is based on its structure, such as its dependence on the oligosaccharide in its molecular weight. In addition, there are two types of oligosaccharid (short or long) 30.

The researcher 31 estimated the molecular weight of Lipopolysaccharide to (70794 Dalton). So, this result was close to the results obtained in this study.

Cytotoxicity of LPS on cell line of breast cancer (mcf-7):

Cytotoxicity of the lipopolysaccharide extracted and partial purified from the local isolate S. enterica serovar Typhimurium S259 was tested in an aquatic phase and phenolic phase which was purified for studying its effect on mcf-7 breast cancer cell line (in vitro).

The results obtained that there was a noticeable toxic effect for different lipopolysaccharide concentrations in phenolic and aqueous phase in the growth of cancerous breast cells mcf-7. That effect starts from the low concentration to toward high concentrations when compared with the control treatment for each phase.

Also the results shown ability of LPS extract for each phases to reduce the density of developing cancer cells. And observed a difference of cytotoxic effect for lipopolysaccharide between the phenolic phase and its cytotoxic effect of aqueous phase.

The statistical analysis results of lipopolysaccharide effect in the phenolic phase showed that there were significant differences between the groups of treatments and the control group. Where the fourth group, represented by concentration 350 µg/ml, recorded maximum of significant differences which was (1700±250) over the other groups comparison with control group (table 2).

As well as the figure (5) shown that the killing rate of cancerous breast cells was 61.67% at the highest concentration of lipopolysaccharide. The figure (6) also shown a gradual variation in the inhibition rates.
of lipopolysaccharide in the phenolic phase, where the inhibition levels in the concentrations 100 μg / ml to 250 μg/ml were as the following: 17%, 30.67%, 46.67%, respectively.

The results also showed significant differences in effect of lipopolysaccharide for both phases on cell line of breast cancer mcf-7, as the results showed the highest significant differences of the phenolic phase than aqueous phase in affectivity on the number and shape of cancer cells line.

The results showed in current study that the cytotoxic effect of lipopolysaccharide was evident in the growth of cancer cells outside the living body during 72 hours of exposure for different concentrations of aqueous and phenolic phases when treated with cancer cell line (mcf-7).

Several studies have accorded to the importance of bacterial toxins in treating various cancer diseases by killing or reducing the growth of cancer cells through changing the cellular processes that control the spread, differentiation and programmed death stages (apoptosis) of living cells.

In several studies have been found that lipopolysaccharide has a toxic effect in reducing the density of developing cancer cells, whether in vitro or in vivo through the introduction of the cancerous cell in the stages of programmed death (apoptosis). The researcher indicated that the lipopolysaccharide enhance the programmed death of breast cancer cells in humans, which inhibits receptors known as TLRs (Toll-like receptors) that have been found to be linked to the development of breast cancer.

Figure (4): Molecular weight of LPS for both aquatic and phenolic phases by using gel filtration chromatography (Sphacryl S – 200) the column dimensions was (1.5 x 70 cm) and elution was done with phosphate buffer saline pH 7.2 at flow rate 34 ml/h
Table (1): Standard protein and standardization of LPS from *S. enterica serovar Typhimurium* for both aquatic and phenolic phases according to the ratio of Void volume and Elution volume (Ve/Vo) ratio.

<table>
<thead>
<tr>
<th>Standard protein and purified LPS</th>
<th>Molecular weight (KD)</th>
<th>Ve / Vo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>232</td>
<td>1.2</td>
</tr>
<tr>
<td>Arginine deaminase</td>
<td>143.548</td>
<td>1.58</td>
</tr>
<tr>
<td>(GTF)glucotransferase</td>
<td>58.2</td>
<td>2.3255</td>
</tr>
<tr>
<td>pepsin</td>
<td>34.5</td>
<td>2.75</td>
</tr>
<tr>
<td>LPS in aquatic phase</td>
<td>71.614</td>
<td>2.142</td>
</tr>
<tr>
<td>LPS in phenolic phase</td>
<td>93.325</td>
<td>1.904</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>67</td>
<td>2.15</td>
</tr>
</tbody>
</table>

Table (2) Effect of lipopolysaccharide in its aqueous and phenolic phases on breast cancer cell line (mcf-7)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Control</th>
<th>Group 1 100 µg/ml</th>
<th>Group 2 150 µg/ml</th>
<th>Group 3 250 µg/ml</th>
<th>Group 4 350 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic</td>
<td>10000+0a</td>
<td>6166+642b</td>
<td>4666+480c</td>
<td>3066+348d</td>
<td>1700+250e</td>
</tr>
<tr>
<td>Aqueous</td>
<td>10000+0a</td>
<td>5333+520b</td>
<td>4100+503c</td>
<td>2400+472d</td>
<td>1733+250d</td>
</tr>
</tbody>
</table>

Different letters mean significant differences between groups at a probability level (P <0.05) depending on the X² test.

Figure (5): Cytotoxicity of LPS (phenolic phase) in breast cell line (mcf-7)
Also\(^{(34)}\) found that exposing the pancreatic cancer cell line to lipopolysaccharide reduced its growth by approximately 50\% by stopping the process of DNA replication at the G1 stage during the life cycle of the cell.

Another study indicated that lipopolysaccharide can stimulate killing of cancer cell by enhancing the immune response through stimulating transcription of encoded genes of proteins which responsible for releasing cytokines that are associated with cyclooxygenase-2 cyclooxygenase that have a role in inhibiting cancer cells\(^{(35)}\).

The current study were consistent with\(^{(36)}\), where he found a cytotoxic effect of lipopolysaccharide in the cell line of cervical cancer when used in high concentrations and the rate of killing of cancer cells were depended on the concentration of LPS.

The study also agreed with\(^{(37)}\) who found that a cytotoxic effect of lipopolysaccharide on esophageal cancer and oral cells when used high concentrations of LPS.

The results also were agreement with\(^{(38)}\), who analyzed the cytotoxicity of lipopolysaccharides by treating the lung cancer cell line - NCI - H69 with different concentrations, including: 100, 150, 250 \(\mu\)g/ml. His results showed A gradual reduction in the growth density of lung cancer cells. He also found that the lowest density of the cancer cells was 48.88\% at a concentration of 250 \(\mu\)g/ml of LPS extract, which was closed result to the present study.

Among the total metabolic processes resulting from the transformation of the normal cell into a cancerous cell is the formation of Free radicals in large quantities\(^{(39)}\). It is believed that the interaction of free radicals with DNA can cause genetic mutations that increase the risk of infection of cancer.

Addition to the oxidative stress reduces programmed cell death and increases proliferation, and growth of cancer cells\(^{(40)}\). Therefore, lipopolysaccharide may selectively remove free radicals and thus adversely effect on the growth and proliferation of cancer cells. Also, there are some active factors in cancer cells known as the (nuclear transcription factor) that have a fundamental role in organizing the cell cycle by coding about cytokines and other growth factors which necessary for cell life, as the presence of these factors increases the resistance of the cancer cells to chemotherapy, so inhibiting these factors will lead to an imbalance in increasing the density of the
developing cells and thus entering the cell to the stage of programmed death (apoptosis) and improving the ability to treatment of cancer disease 41.

Also, 42 provided the ability of LPS as potential anticancer agent in breast cancer that ability inducing apoptosis and could be used LPS for wide biomedical applications and could be offer new drug instead of chemotherapy in treatment of various types of cancer disease.

**Conclusion**

We concluded from this study that LPS have an obvious cytotoxic effect on the line of breast cancer cells in different concentrations Also, we concluded that the LPS of *S. enterica* serovar *Typhimurium* has cytotoxic properties which can be used in development of drugs for treatment of cancer disease.

**Conflict of Interest:** Nil.

**Funding:** Self-funding

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Detection of Antiseptic Resistant Genes in Colistin-Resistant Pseudomonas aeruginosa and MDR Klebsiella pneumoniae

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Abstract

Objectives: The aim of this study was to detect the existence of developments in resistance to biocide of Pseudomonas aeruginosa resist of colistin and in multi-drug resistance Klebsiella pneumonia in the hospital environment. Materials and Methods: The study included 25 isolates of K. pneumoniae and 30 isolates of P. aeruginosa. Isolated from different clinical and environmental samples in Baghdad hospitals. Antibiotic sensitivity tests and their susceptibility to multiple antibiotic resistance and sensitivity tests were studied for the most commonly used antiseptics at the preventive level (benzalkonium chloride). The test was carried out using the micro dilution broth method, following Institution of Clinical and Laboratory Standards guidelines, PCR was performed for detection of blaTEM, blaSHV, blaCTX-M, qacC/D, qacΔE1 and qacE beta lactamase and antiseptic genes. Results: A high rate of multiple resistance to the most used antibiotics was observed, so the rate of resistance to all antibiotics that was used was 16.0% of P. aeruginosa and 4% of K. pneumoniae possesses comprehensive resistance to all antibiotics that were used and the resist of colistin in P. aeruginosa was 36%. The prevalence of ESBLs was 36.0% and 48.0% of Paeruginosa and K.pneumonia respectively, in addition to their strong ability to form biofilms 80% in Paeruginosa and 94% in k.pneumoniae and their ability to resist Antiseptics. The percentage of resistance to antiseptic benzalkonium chloride showed the highest concentration of Paeruginosa was 33.3% and K. pneumoniae 37.5%. The result of ESBL and antiseptic genes detection clarify, the percent of production genes were (10%), (40%) blaTEM; (6.66%), (56%) blaSHV; (33%), (64%) blaCTX-M; (70%), (44%) qacC/D; (80%), (56%) qacΔE1 in Paeruginosa and K.pneumoniae respectively and no any isolate carried qacE gene. production of extended spectrum β-lactamase genes in addition to their strong ability to form biofilms 80% in p.aeruginosa and 94% in k.pneumoniae and their ability to resist an antiseptics. The percentage of resistance to antiseptic benzalkonium chloride showed the highest concentration of P. aeruginosa was 33.3% and K. pneumoniae 37.5%, the result of beta lactamase and antiseptic genes detection clarify PCR was performed for detection of blaTEM, blaSHV, blaCTX-M, qacC/D, qacΔE1 and qacE beta lactamase and antiseptic genes. Conclusion: Our observations indicate that there is a significant correlation between the ability of bacteria to resist multiple antibiotics in addition to their ability to resist the most commonly used antiseptics, due to their physiological nature and increased virulence factors.

Keywords: Antiseptic, Antibiotic Resistant genes, Pseudomonas aeruginosa, Klebsiella pneumoniae

Introduction

Hospital infection represents one of the most common challenges facing health systems in the developed world such as Health Care - Associated Infection (HCAI) as the number of hospitals which acquired infections (HAI) is increasing dramatically worldwide, especially due to the emergence of multidrug-resistant bacteria (MDR). MDR isolates spread is easily observed in hospitals settings, and are seen specially in a patient while under medical care in a hospital or other healthcare facility. This infection can occur during health care provision for other illnesses and even after patients have discharged the disease, an occupational infection may form among medical personnel. There are two types of bacteria that are common in nosocomial and respiratory infections, Klebsiella pneumoniae and Pseudomonas aeruginosa that most often cause human nosocomial infections.
K. pneumoniae and P. aeruginosa have the ability to form biofilm on medical devices, such as catheters and ventilators. In some cases, pneumonia is excessive and very violent and can spread and affect healthy people, causing life-threatening and often community-transmitted infections, along with pyogenic liver abscess, meningitis, necrotizing fasciitis, endometriosis, and acute pneumonia.

Colistin is a perfect antibiotic against Gram-negative bacteria, the most important of which is Pseudomonas aeruginosa. Where it is effective against the outer membrane of bacteria that are negative for the Gram stain, specifically anionic lipopolysaccharide (LPS). Of concern is the high rate of resistance to Pseudomonas aeruginosa to colistin, as this is explained by the bacteria’s possession of two main mechanisms of resistance to colistin in Gram-negative bacteria, mutation and adaptation, as the resistance resulting from mutations is the increase in gene expression on the reflux pumps, Adaptive is the ionic change of the ions components present in the cell membrane.

Antiseptics and disinfectants are widely used in hospitals and other healthcare settings to prevent infection and reduce the chances of contracting diseases and epidemics, despite the widespread use of disinfectants, a high rate of microbial contamination has recently been observed. This may be due to the development of multiple virulence factors and resistance to both antibiotics and disinfectants due to its ability to adapt to the indiscriminate use of near-lethal concentrations of disinfectants.

K. pneumoniae and P. aeruginosa are dangerous micro-organisms, capable of growing on solid, non-porous surfaces, in addition to their possession of multi virulence factors, the most important of which is their ability to form biofilms. There is a wide range of active chemical agents (or “biocides”) in these products, and many have been used for hundreds of years for sterilization, disinfection and preservation.

Biocides have a wider range of effect compared to antibiotics, as antibiotics have specific intracellular targets in addition to their specialization, while biocides have comprehensive and non-specific effectiveness. Nevertheless, the widespread use of disinfectants has led to pathogens acquiring resistance factors that may be common with antibiotic resistance and what is known as cross resistance. It is important to note that many of these biocides can be used alone or with a variety of products, which differ widely in their activity against microorganisms. Antiseptics differ according to their effectiveness towards the vital cell. Some of them target cell membranes, plasma membranes, nucleic acids, or they may be oxidizing agents. Therefore, hospital disinfection has a major role to play in controlling health care-related. Disinfectants play an essential role in controlling infection and preventing the transmission of infectious pathogens in hospitals. The aim of the study is to detection the antiseptic resistance genes and the extent of their prevalence in multidrug resistant clinical and environmental bacterial isolates in hospital.

Materials and Methods

Samples Collection:

Fifty-five samples were collected from clinical and environmental sources, 30 isolates of Pseudomonas aeruginosa and 25 isolates of Klebsiella pneumoniae, initially diagnosed in hospitals. The samples included swabs for burns, wounds, urine, sputum, and swabs from intensive care rooms, operating rooms, main operating rooms, patient halls, and the children’s protection hall, in addition to swabs for fluid withdrawal devices, endoscopes, surfaces and sinks for preterm infants, which were collected during the period from July to October 2019.

Bacterial Isolates:

Primary diagnosis based on morphological characteristic of the colonies that included colony shape, texture, color and edges dependently on bacterial growth on the MacConky agar and blood agar. All isolates were identified using conventional biochemical tests and vitek 2 system.

Antibiotic Susceptibility Test:

The susceptibility of isolates to different antibiotics was tested using the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guidelines. Using antibacterial agents included: gentamicin (GM), amoxi clav (AUG), amikacin (AK), ceftriaxone (CRO), levofloxacin (LEV), deoxycycline (DXC), piperacillin tazobactam (PTZ), ceftazidime.
(CAZ), cefazolin (KZ), aztreonam (ATM), tetracycline (T), cefepim (EFEP) and colistin (CO). The bacterial culture was carried out using Muller-Hinton agar medium (HiMedia, India) and the bacterial suspension prepared with a dilution standard corresponding to the McFarland standard, after which the cultivated plates were incubated at 35 °C for eighteen hours.

Detection ESβLs by using Vitek-2 system:

Detection Phenotypic of ESβLs producing isolates were also done by Vitek-2 system by using sensitivity of antibiotic test Number (AST-GN69) card according to the manufacturer’s instructions.

Detection of Biofilm formation by Micro titer plate assays:

In this study, isolates of *P. aeruginosa* and *K. pneumoniae* were examined for their ability to form biofilms. A micro titer plate was used according to the method described by 17, 18. Twenty μl of the bacterial suspension was taken from an overnight culture to inoculate the micro titer wells containing 180 μl of Brain Heart Infusion (BHI) broth with 1% sucrose. Thus, the control wells which contained 200 μl of BHI broth with the bacterial suspension. Then the micro titer plate was closed and covered with Para film during incubation at 37 °C for twenty-four hours. Unlinked bacterial cells were surely removed by washing the wells three times with PBS (pH 7.2), then kept at room temperature for fifteen minutes in order to dry, then 200 μl of crystal violet (0.1%) was added to the wells for a period of time. Fifteen minutes. After removing the crystal violet solution, the wells were washed three times with PBS (pH 7.2) to remove the unbounded dye and allowed to dry at room temperature, after which 200 μl of 95% ethanol was used for the purpose of extraction. And in Final, the optical density value of each well is deducted by an ELISA reader at 630 nm absorption degree.

Determination of Minimal Inhibitory Concentration (MIC) for chemical antiseptics:

Fill all wells in a 100 μl micro titer plate of BHB, after that 100 μl of activated isolates suspension after adjusting turbidity to 1 x 10^8 cfu / ml as 0.5 McFarland with normal saline is added to the wells in a micro titer plate. and then the detergent concentration is then diluted using the two-prong dilution method until we obtain a series of dilutions with pre-added BHB from high to low concentration distributed in wells (A to H) in wells (1-10). The row of wells A12- H12 is considered as a positive control. Whereas (A11 - H11) which is a passive control. After incubation for 18 hours at 37 °C, add 60 μl of resazurin sodium to each well in the dish and leave it for (2-4) hours in the incubation to observe the color change 19.

Molecular detection of β-lactamase and antiseptic genes using PCR technique:

All isolates were submitted to PCR technique to detection for ESBLs and antiseptic genes; blaTEM, blaSHV , blaCTX-M , qacC/D, qacΔE1and qacE by using Specific primers (table 1) 20. DNA of isolates were extracted by using a commercial purification system (Genomic DNA Purification Kit) and PCR was used to amplify genes. PCR mixture was set up for each gene alone in a total volume of 25 μl included 12.5μl of Go Taq Green Master Mix, 1.5 μl of each primer (10 picomole/ μl) and 4 μl of template DNA. The volume remaining was completed with sterile nuclease free water PCR products were detected by agarose gel electrophoresis. A DNA marker (Promega/USA) was run with each gel, and the genotype was determined by the size of the amplified produce.
Table 1: The sequences of ESβL and antiseptic primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences (3'-----5')</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>qacΔE1</td>
<td>F: AATCCATCCCTGTGGTT R: CGCAGCGACTTCCACGATGGGAT</td>
<td>155</td>
<td>Zou et al., (2014)</td>
</tr>
<tr>
<td>blaTEM</td>
<td>F-ATGAGTTACACATCCTGTT R- TTACCAATGCTTAATCAGTGAG</td>
<td>861</td>
<td>Szabo et al., (2005)</td>
</tr>
<tr>
<td>blaSHV</td>
<td>F-ATTTGTGCTTCTTACTCGC R-TTATGGCGTTACCTTGACC</td>
<td>1051</td>
<td>Szabo et al., (2005)</td>
</tr>
<tr>
<td>blaCTX-M</td>
<td>F- CGCTTTTCGATGTCAG R- ACCGCGATATCGTTGAG</td>
<td>544</td>
<td>Szabo et al., (2005)</td>
</tr>
</tbody>
</table>

Results and Discussion

In this study 55 samples were collected from clinical and environmental sources. All samples were transferred to the laboratory by transport media, then cultured on MacConkey agar and blood agar and incubated for 18-24 h at 37 °C. Then the isolates were diagnosed and confirmed by using a VITEK-2 pressurized system according to the manufacturer’s instructions (Biomerieux / France). The study included resistance of these isolates to antibiotics and antiseptics in addition to their possession of virulence factors and their ability to form biofilms 21. *Klebsiella pneumoniae* is a type of Gram-negative bacteria and can cause different types of health care-related infections. *Klebsiella* infection usually occurs among patients who are receiving treatment for other conditions. Patients whose care requires devices such as ventilators (ventilators) or intravenous tubes for drugs and urinary catheters 22. Patients who take long courses of some antibiotics are more likely to develop a *Klebsiella* infection 23. Antibiotic susceptibility testing was performed *P. aeruginosa* and *K. pneumoniae isolates*, results showed that isolates (36%) of *P. aeruginosa* were resistant to colistin, all isolates were resistant to amoxiclav, tetracycline and doxycycline (100% each). In addition, 46% of the *P. aeruginosa* isolates were resistant to gentamicin and to tazobactam. In *K. pneumoniae*, the percentage of resistance to gentamycin was 20% and to tazobactam 28%. Cefazolin and aztreonam 90% of *P. aeruginosa* and in *K. pneumoniae* rate of resist to aztreonam was 64% and in Cefazolin was 72% and the same percentage that was in the case of cefazidime. And about the resistance rate in the pseudomonas, it was 50%, for amikacin resistance 40% of *P. aeruginosa* isolates and 16% for *K. pneumoniae* isolates, and for levofloxacin resistance was 53% for the *P. aeruginosa* isolates and 36% for the *K. pneumoniae* isolates, and the cefepime resistance was 46% in all isolates. *K. pneumoniae* showed greater resistance to ciprofloxacin 63% in *P. aeruginosa* and 68% in *K. pneumoniae* as shown in the table 2.

During the past decade, observe increasing in rates of antimicrobial resistance has been recognized worldwide,
and an increased frequency of MDR isolates has also been demonstrated in clinical environment samples. Where the term drug resistance was applied to isolates for which no treatment options were available.

It has been observed during this study that the bacteria communities follow different strategies in antibiotic resistance, which may be physiological or genetic. In terms of clinical isolates, they gain resistance to multiple antibiotics due to patients being exposed to long periods of treatment with these antibiotics, in addition to the irregular use and irregular concentrations of these antibiotics, which led to a gradual adaptation of pathogens to generate defenses against them and one of the most important of these methods is refluxes pumps one of the most important means of multiple antibiotic resistance. While isolates taken from hospital environment and medical devices that indicate contamination of the hospital environment and medical devices with microbes have multiple resistance to antibiotics as their resistance to antibiotics varies according to the source of contamination. If the source of contamination is from the soil, which is the home of bacterial isolates where they acquire self-resistance to antibiotics produced by other bacterial types present in the soil, and the other source is medical waste, which is similar to clinical samples that come from infected patients.

In this study, the bacterial isolates showed their ability to produce extended spectrum beta lactamase included (36.0%) of P.aeruginosa isolates and (48.0%) of K.pneumoniae isolates were ESBLs produced. This is in line with the study where included (42.30%) of P. aeruginosa isolates were positive for ESβLs, and in another study by showed that (46.2%) of K. pneumoniae isolates were positive for ESβLs. This is consistent with what we have reached, and in a comprehensive study made by it was shown that (50%) of Klebsiella sp were positive for ESβLs.

P. aeruginosa and K. pneumoniae were from clinical and environmental sources as MDR and have ability to biofilm form biofilm was assessed by a micro titer plate. The results showed that 80% of P. aeruginosa and 96% of K. pneumoniae were production biofilms, The results were consistent with the results of other studies, in study by results showed (85.63%) of P. aeruginosa producing biofilms. We have shown the similar study reported by Hassan and other K. pneumoniae (64.7%) as high or medium productive biofilms and 40 isolates (35.3%) were identified as poorly produced biofilms. Saifi et al. Reported that the majority of K.pneumoniae (93.6%) were biofilms and only 6.4% were not biofilms. The percentage observed of biofilm formation in clinical samples increased significantly compared to environmental samples. These differences in levels of formation biofilm in clinical samples may be the result of repeated use of drugs, also with high period duration of antibiotic treatment, and excessive use of drugs in the animal and poultry industry that is consumed by humans.

It is worth mentioning that the incorrect use of disinfectants and detergents and the increase of mutagenic bacteria in today’s industrial life. As isolation of clinical source has some selectivity in the formation of biofilms, which is related to the presence of proteins necessary for the growth and biofilms formation associated with the outer membrane, and we note that these membrane proteins need time to adapt to the conditions. It can be seen that the surrounding environment conditions provided in the clinical samples are suitable for them and do not need to be adapted.
### Table 2: Antibiotic Susceptibility of *P. aeruginosa* and *k. pneumoniae*

<table>
<thead>
<tr>
<th>NO.</th>
<th>Antibiotic</th>
<th>Percentage of resistance (%) in <em>P. aeruginosa</em> isolates</th>
<th>Percentage of resistance (%) in <em>k. pneumoniae</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amoxi-clav 30µg</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Cefazidim 30 µg</td>
<td>50</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>Tazobactam 30 µg</td>
<td>46</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Ceftriaxone 10 µg</td>
<td>63</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>Deoxycycline 10 µg</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Amikacin 10 µg</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>Cefepim 10 µg</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>8</td>
<td>Tetracycline 30 µg</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Aztreonam 30 µg</td>
<td>90</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>Levofloxacin 10 µg</td>
<td>53</td>
<td>36</td>
</tr>
<tr>
<td>11</td>
<td>Cefazolin 30 µg</td>
<td>90</td>
<td>72</td>
</tr>
<tr>
<td>12</td>
<td>Gentamycin 10 µg</td>
<td>64</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>Colistin 10 µg</td>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>

Antibiotic resistance and biofilm formation among hospital pathogens is a risk that is difficult to eliminate over time. Producing lactamase and spread among bacterial pathogens adversely affects the possibilities of antibiotic therapy, which is worth noting biocides play an important role in preventing and controlling hospital-acquired infections. The lowest antimicrobial agent for the minimum inhibitor concentration (MIC) is usually tested, and the purpose of this test is to study and find out the sensitivity of bacteria to the antiseptic and to know the lowest concentration that may affect them. The results of the study showed the minimum inhibitory concentration (MICs) for Benzalkonium chloride, were 33.3% of *P. aeruginosa* and 37.5% of *K. pneumoniae* isolates had MIC 40000 µg/ml (4% ), whereas 62.5% of *P.aeruginosa* and 50% *K. pneumoniae* isolates had MIC 20000 µg/ml (2% ), while 66.6% of *P.aeruginosa* and 62.5 % *K. pneumonia* isolates had MIC 10000 µg/ml (1%),fig.1

![Figure (1): Dilution to concentration of Benzalkonium Chlorid](image-url)
The emergence of this resistance is related to the ability of bacteria to adapt to these antiseptics through the acquisition of virulence factors represented in the formation of the cell wall and reflex systems, in addition to their acquisition of antiseptic resistance genes, which may have close relationship with the antibiotic resistance genes, and this resistance has increased specifically in the recent times. by using disinfectants with near-fatal concentrations of the microbe, it gave the opportunity to coexist and develop for resistance and survival.

By using PCR The results of detection of extended spectrum β-lactamase and antiseptic genes showed the presence of qacΔE shows in (80%) of P.aeruginosa and in (56.0%) of K.pneumoniae isolates; qac C/D shows in (70.0%) of P.aeruginosa and in (44.0%) of K.pneumoniae; bla _CTX_ in (33.0%) of P.aeruginosa and in (64.0%) of K.pneumoniae isolates; bla _SHV_ in (6.66%) of P.aeruginosa and in (56.0%) of K.pneumoniae ; bla _TEM_ shows in (10.0%) of P. aeruginosa and in (40.0 %) of K.pneumoniae and no any isolate carried qac E genes, as shown in the table 3.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. Clinical isolate</th>
<th>No. Environmental isolate</th>
<th>Genes %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.aeruginosa</td>
<td>16</td>
<td>11</td>
<td>qacΔ E 80</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>8</td>
<td>qac C/D 70</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>bla <em>CTX</em> 33</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>bla <em>TEM</em> 10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>bla <em>SHV</em> 6</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>14</td>
<td>2</td>
<td>qacΔ E 56</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>qac C/d 44</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1</td>
<td>bla <em>CTX</em> 64</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1</td>
<td>bla <em>TEM</em> 40</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0</td>
<td>bla <em>SHV</em> 56</td>
</tr>
</tbody>
</table>

In a study conducted in Iraq by revealed that all _P. aeruginosa_ isolates (100%) carry bla _CTX_. Bokaeian et al stated that the percentage of bla _TEM_ gene was (100%) in _P.aerugenosa_ Iran . Mohamed et al reported that (90%) of _K.pneumoniae_ isolates carried bla _TEM_ and bla _CTX_ genes respectively in Egypt, and another study was conducted in Egypt, and reported that the percentage of bla _CTX-M_ is (53.3%) in _K. pneumoniae_ isolates in a. The study by found that the percentage of the bla _TEM_ gene is 71.7% and the bla _CTX-M_ gene is 99.2% in _K. pneumoniae_ isolates. Excessive, semi-fatal and intense use led to the emergence of bacterial isolates that are clearly resistant to the dangerous concentrations of disinfectants that are not only resistant to antibiotics. Special genes responsible for host activities such as multiple flow systems and the possibility for these genes to be transmitted between bacterial strains through direct contact or via mobile plasmids.

Hilal et al. revealed that qacEΔ1 and qacE gene replication and their association with antibiotic and biocide resistance in _Pseudomonas aeruginosa_ isolates in Egypt. It was found that the percentage of qacE1 gene was 57.8% in MDR isolates and 13.4% in bacterial isolates. Sensitive to several drugs, while the secreted mucilage was only present among the multidrug-resistant _P. aerugenosa_, while conducted by Amazonian 2014 in a study Maliocytes by , the percentage of quaternary ammonium complex resistance genes (QACs) in _P. aeruginosa_ was (14.28%) Contained the qacC / D gene while the qacEΔ1 gene was 100%

In another study by , the qacEΔ1 gene was detected in 48% of an isolate. Eighty-eight percent of the poly-resistant isolates carried the qacEΔ1 gene, while 35% of the non-resistant isolates were positive for this gene, and multiple resistance was well correlated with its presence.
Among the isolates tested, more than one study has been reported on *K. pneumoniae* by And Abu Zayd and Ames in the UK showed that susceptibility to disinfectants was decreased due to the presence of the *qacΔE1* and *cepA* genes.

**Conclusion**

Our observations indicate a significant correlation between the ability of bacteria to resist many antibiotics in addition to their ability to resist the most common antiseptics due to their physiological nature and increased virulence factors, noting the high incidence of *Pseudomonas aeruginosa* against colistin and the emergence of multiple antibiotic resistance isolates of *Pseudomonas aeruginosa* and *Klebsiella Pneumonia* and high incidence of genes responsible for beta-lactam resistance and antiseptic resistance.

**Funding:** Self-Funding.

**Author Conflict:** Nil

**Ethical Clearance:** Taken from education committee of biology department, college of science, Mustansiriyah University, Baghdad, Iraq.

**References**


Detection of HMA5, PCs and MT2 Genes Expression in *Vicia faba* Under Heavy Metal Stress Using Quantitative Real-Time PCR

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Abstract

In order to achieve phytoremediation during agricultural production, it is essential to identify high genotypes that are able of accumulating many types of heavy metals not in the edible parts which have significant nutritional value but in the non-edible parts. This study conducted to estimate the heavy metal ATPases 5 (HMA5), Phytochelatins synthase (PCs) and metallothionein 2 (MT2) genes expression in plant *Vicia faba* in response to an elevated concentration of copper and zinc in nutrient media. Using Quantitative Real-Time PCR (RT-qPCR) technique, the results of hydroponic culture methods with high concentrations of copper (75 µMol / L) and high concentration of zinc (500 µMol / L) showed high expression level for the three genes of *Vicia faba* plant compared with control. Established that the expression of the genes under the influence of copper ion was higher than the expression under zinc ion influence. Besides that, gene expression increased with increased exposure time to zinc ion, also in the case of copper ion exposure time, all genes expression slightly increases with increased exposure time. In response to excess copper and zinc, an increase in the expression of genes (HMA5, PCs and MT2) involved in plant protection, providing the possibility of its transfer from the cytosol to the apoplast demonstrate that this plant might be useful for phytoremediation of moderately polluted areas with copper or zinc.

Keywords: *Vicia faba*, HMA5, PCs, MT2, copper, zinc, RT-qPCR.

Introduction

Heavy metals can be a major problem for different organisms as may be reactive with several chemicals essential to biological processes. Many chemical and physical methods were used for soil reclamation and remediation, however, these techniques usually required high maintenance costs and may lead to secondary pollution.

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Absorption and transportation are crucial mechanisms of plant tolerance to heavy metals which can be performed by heavy metal-associated isoprenylated protein (HMA). The phytochelatins (PCs) are also linked to metalloids with heavy metals to produce PC-metal complexes which are very stable and have less toxicity rather than free metal ions present in the cells. Under natural conditions, PCs are actively involved in the degradation of various glutathione conjugates in comparison to other metalloids. Metallothioneins (MTs) bind to different metal ions due to high affinity of the sulfur molecule in the thiol group of cysteine and exert a major role in the detoxification of heavy metal stress.
In the Mediterranean Basin and Arab countries, Faba bean (Vicia faba) considers as one of the most important legume crops, which is an efficient nitrogen fixer. Faba bean is a plant that had the ability to grow in various climatic zones. Additionally, it can be consumed throughout the year, as it can be utilized in both raw and processed forms. For a human, it is mostly the seed grain that is consumed, whereas the pods are used as animal feed. The plant pods have micro and macro compounds, however, they can a good source of functional phytochemicals. The nutritional importance of Faba bean is lying behind a prominent high protein that offers a valuable amount of energy. This legume plant has also therapeutic potentials as it provides the precursor to a drug used in Parkinson’s disease treatment. Parts of faba bean plants and its processing products such as grains, hulls, and flowers considered as good source of fiber and non-nutrient secondary metabolites which could be salutary to human health. Many reports showed that high protein foods particularly animal-based have a high probability of causing intestinal problems in the long term of use, especially cancer, due to a lack in antioxidant compounds and an abundance in dangerous metabolites. Enhancements of the quantity and quality of food proteins could be done by a combination of legume plants such as faba bean with different plant-based foods. The consumption of faba bean seeds provides some essential amino acids required for normal growth and repair of damaged tissues. Further research that can lead to a reduction in the current extent of yield variability is needed, thus faba bean may prove to be a key component of future arable cropping systems where declining supplies and high prices are likely to constrain the affordability and use of fertilizers. As the development of food production is in continuously processes, and due to environmental and dietary beneficial of faba bean, it could be grown in the market within the next years, and become economical and valuable agricultural products such as soybean.

Recent studies indicated the ability of Vicia faba to tolerate the elevated concentration of heavy metals such as copper. The metal translocation in plants mainly depends on plant species and type of metals. Negative effect of oxidative stress may results either from the increased concentration of essential metals (micronutrients) like Zn, Cu and Ni or nonessentials such as Pb and Cd. Heavy metals are common soil pollutants. Therefore, the effectiveness of plant defense system (antioxidants) in plants should be crucial to clarify the mechanisms of plant tolerance to heavy metals. Production of tress concentration of different metabolites, such as amino acids (prolin, ascorbic acid and histidine) and peptide (e.g., glutathione GSH) or phytochelatins (PC), could be essential for the mechanisms of defense against heavy metals effects. These low molecular weight antioxidants detoxify oxygen free radicals. Also formation of metals nontoxic complexes by binding with nonprotein compound but rich in -SH groups which is an important factor to perform plant’s tolerance to heavy metals ions.

Based on the above and due to the importance of Faba bean in detoxification of heavy metal ions, this work conducted to investigate the expression of HMA5, PCs and MT2 genes in plant Vicia faba.

**Material and Methods**

**Treatment of seeds**

Local Faba bean seeds were disinfected by washing thoroughly with tap water for 15 minutes, sterilized with 2 % v/v sodium hypochlorite (Clorox) for another 15 minutes and then washed extensively with sterilized distilled water.

**Sowing and cultivation the initial stage of seedlings**

Disinfected seeds were sown on the surface of moist perlite in plastic containers (40 cm x x 30 cm x 8 cm) with holes at the bottom, placing the container on a tray. To maintain the desired humidity the top of the container closed with a glass plate and removing it only after sprouting.

**Seedlings replantation in hydroponic system**

At the age of 14 days, the plants removed from the perlite by using a spatula without damaging the roots. Then the roots washed in a small volume of water to remove the perlite particles. The plants are then cultivated in 1-liter pot, at a rate of three plants per pot. Aeration and mixing of the nutrient solution carried out by a continuous and uniform supply of air. In water culture plants grown in a growth chamber at the temperature range of 23-25 / 18-20 °C (day/night), 16:8 h light: dark photoperiod. For growing in hydroponic, an MS
medium was adopted \textsuperscript{29,30} and pH adjusted to 5.8.

Experimental conditions

In the experiments used 5 to 6-week old plants with 3-4 fully developed leaves. Exposure started by the introduction of CuSO\textsubscript{4} to the culture medium at concentrations of 75 µMol \textsuperscript{18} and ZnSO\textsubscript{4} at a concentration of 500 µMol \textsuperscript{31}. Changing the culture medium performed every 5 – 7 days. As a control, plants grown on standard MS medium.

Gene Expression Study

Gene expression for \textit{HMA5}, \textit{PCs} and \textit{MT2} genes were determined using RT qPCR technique by comparative Ct values of specific amplification for each gene to measure the level of gene transcription (mRNA level) \textsuperscript{32}. The Ct of 18srDNA was used as an endogenous control for calibrating the Ct values of other genes \textsuperscript{33}.

RNA Extraction

Total RNA was extracted from the 5gm of fresh leave tissue (after 1 and 2 weeks of exposure to heavy metals) with the use of TRIZOL® reagent (Invitrogen, USA) according to the manufacture’s protocol. For lysis, 1 ml of Trizol solution was added to each sample. For three phases separation, 0.2 ml of chloroform was adding then tubes centrifuge for 10 min at 12000 rpm. RNA samples then concentrated using isopropanol followed washing using 70% ethanol. Finally, RNA pellet diluted using nuclease-free water. Quantus Fluorometer (Promega, USA) was used to determine the concentration of extracted RNA. For 1 µl of RNA, 199 µl of diluted QuanutyFlour dye was mixed. After 5 minutes of incubation at room temperature in a dark place, RNA concentration values were detected.

Quantitative Real-Time PCR (RT–qPCR)

All RT-qPCR studies were designed to comply with the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines where applicable or practical. RT-qPCR reactions were carried out with a mic real-time PCR system using GoTaq® 1-Step RT-qPCR System (Promega). Each 10 µl reaction volume contained 1 µl of RNA, 5 µl (2X) GoTaq® 1-Step RT-qPCR, 3 µl dH2O, and 0.5 µl (10 µM) of each primer. The sequences of the selected genes were found in the American National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) nucleotide sequence database. The selection of primers for the coding part of the target genes was carried out using the VectorNTI 9.0.0 program. The primer designed to amplify mRNA from:

\textbf{HMA5}, \textit{5-GACAACGACGATTCTCTGAGTAA-3}

\textbf{F:} 5-TAACACAAGCAGCACAAGTCAT-3,

\textbf{PCS}, \textit{F:} 5-ATCAGACCACCATTGACGACTT-3

\textbf{R:} 5- GAACTCACAAGACGAGGAACATCT-3,

\textbf{MT2}, \textit{F:} 5-GTCTTGCTGTGGAGGGAAACTGT-3

\textbf{R:} 5- GGGTTGCACTTGCAGTCCAGAT-3)

\textbf{18s rRNA} gene as endogenous control, \textit{F:} 5-GAGTGATGTGCCAGACCTAGGAATT-3

\textbf{R:} 5- ATGCTGATCCCGGATTACAGC-3.

The reaction conditions included cDNA synthesis step of 37°C for 15 min. followed by an initial denaturation step of 95°C for 30 s, 40 cycles of 95°C/20 s, 60°C/30 s and 72°C/30 s. The dissociation curve was obtained by heating the amplicon from 65 to 95°C. A non-template control was also included for each gene. The primer annealing temperature was calculated using the Vector NTI Suite 9 program \textsuperscript{34}.

Results

The genes expression at the transcription level was evaluated by estimating change in folding level of mRNA transcripts using the RT-qPCR technique (Table 1).
Table 1 The estimated values of HMA5, PCS and MT2 genes expression under the influence of copper (75 µMol./L) and zinc (500 µMol./L)

<table>
<thead>
<tr>
<th>Time</th>
<th>Groups</th>
<th>Ct 18sRNA</th>
<th>Ct HAM5</th>
<th>Folding</th>
<th>Ct PCS</th>
<th>Folding</th>
<th>Ct MT2</th>
<th>Folding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Control</td>
<td>26.4</td>
<td>27.90</td>
<td>1.00</td>
<td>20.90</td>
<td>1.00</td>
<td>21.20</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CuSO4 75 µMol.</td>
<td>27.5</td>
<td>27.80</td>
<td>2.30</td>
<td>21.00</td>
<td>1.37</td>
<td>21.70</td>
<td>1.52</td>
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<tr>
<td></td>
<td>ZnSO4 500 µMol.</td>
<td>26.2</td>
<td>27.00</td>
<td>1.62</td>
<td>20.10</td>
<td>1.04</td>
<td>21.00</td>
<td>1.07</td>
</tr>
<tr>
<td>2nd week</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Control</td>
<td>27.5</td>
<td>29.30</td>
<td>1.00</td>
<td>20.90</td>
<td>1.00</td>
<td>22.20</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CuSO4 75 µMol.</td>
<td>28.2</td>
<td>28.50</td>
<td>2.83</td>
<td>21.40</td>
<td>1.68</td>
<td>22.20</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>ZnSO4 500 µMol.</td>
<td>27.3</td>
<td>28.20</td>
<td>1.87</td>
<td>21.00</td>
<td>1.19</td>
<td>21.70</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Folding = $2^{-\Delta\Delta Ct}$

$\Delta Ct = Ct (gene) - Ct (control)$,

$Ct = (\text{gene value from RT qPCR}) - (\text{18sRNA value from RT PCR})$.

The expression activity assessed at the level of the total content of individual transcripts at 7 and 14 days of growing plants in MS media with a high concentration of CuSO₄ and ZnSO₄ separately as compare with plants that grow without heavy metal stress as control.

Copper vs. zinc as stress type

Results shown in Fig.1 indicate that HMA5 gene exhibited a constant activity, and its expression was significantly higher in response to copper than zinc ions. On the other hand, PCS and MT2 gene exhibited a varied activity, and its expression was slightly higher under the influence of copper than zinc.

HMA5 vs. PCS and MT2 as the investigated genes

Results shown in Fig.1 indicate that HMA5 gene exhibited a constant activity, and its expression was slightly higher than other two genes (PCS MT2) under influences of copper and zinc at the 1st week and the 2nd week of exposure.

1st vs 2nd week as exposure time.

HMA5 gene exhibited a constant expression enhanced with longer exposure to copper and zinc, that the expression level increased significantly with increased exposure time. While PCS and MT2 genes showes increas in gene expression under influence of copper and zinc comparing with control but there were no significant diffrenece between the level of expression at different exposure time (Fig.1).
Discussion

The resistance of *Vicia faba* plant to the toxic effect of copper and zinc ions could be related to changes in the expression of *HMA5*, *PCs* and *MT2* genes that involved in the regulation of intracellular homeostasis of the plant.

The obtained results showed that in all tested samples, the differences in the expression of the studied genes under the different heavy metals influence are mild. At the same time, manifested activity with an excess of heavy metals in the medium confirms their participation in the protective reactions of the investigated plants. This applies to the participation of membrane transporter gene *HMA5* and the chelation of copper and zinc ions with the participation of phytochelatin synthase gene (*PCs*) and metallothioneins genes (*MT2*) carrying the excess copper and zinc ions from the cell to the extracellular space (apoplast). The activation of expression of these genes encoding a chelation and membrane transporter, transferring excess copper or zinc from the cytosol into the cell wall, where its detoxification is carried out by binding with pectins and hemicellulose. Activation of these genes expression in leaves of *Vicia faba* plants may be one of the reasons for the increased resistance of *Vicia faba* to the toxic effect of excess copper or zinc in the medium. This can serve to protect the plant from the toxic effect of increased content of CuSO₄ in the medium.

Conclusions

The increased activation of *HMA5*, *PCs* and *MT2* genes expression may indicate the formation of stress-protective mechanisms of plants from the toxic effect of high concentrations of copper ions in the environment.

Under the influence of excess copper or zinc, an increase in the expression of genes involved in plant protection was established, providing the possibility of its transfer from the cytosol to the apoplast *HMA5* which exhibit a higher expression level than *MT2* and *PCs* genes.

*Vicia faba* plant is potentially useful for phytoremediation of moderately polluted areas with copper or zinc ions.

**Conflict of Interest:** We declare that there is no conflict of interest.

**Source of Funding:** None.

**Ethical Approval:** Obtained from the college ethics committee.
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Detection of Tn916 Conferring Tetracycline Resistance in Clinical Isolates of *Streptococcus pyogenes*

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Abstract

Background: During the past twenty years, tetracycline resistance average has increased in *Streptococcus pyogenes* in many countries. Pneumococcal resistance to erythromycin and tetracycline is associated with the insertion of the *erm*(B) into the transposons of the Tn916 family. To get datum that may be beneficial in resolving the diffusion of antimicrobial resistance, therefore, we can be specified antibiotic resistant genes and their corporation with mobile genetic elements. This study was proceed to explore the genetic regulation of Tn916-carrying *tet*(M) in clinical isolates of *S.pyogenes*. Methods: A twenty two of *S.pyogenes* isolates were assemble from patients suffering from upper respiratory infection, and the susceptibility of these isolates to tetracycline antibiotics was examined. Molecular detection of Tn916 was carried out by employ certain primers to amplify *tet*(M) gene in each isolates.

Results: The results appeared that the resistance of the tetracycline group was 68.1%, 54.5%, 36.3% and 31.8% for minocycline, tetracycline, oxytetracycline and doxycycline, respectively. Genetic analysis showed that Tn916 was detected in eight of the *S.pyogenes* clinical isolates resistant to tetracycline. Conclusions: Our findings suggest that clinical isolates of *S. pyogenes* harboring a copy(s) of Tn916 conferring tetracycline resistance. One possible explanation for resistance to tetracycline in these isolates is due to *tet* gene, which was most likely located on Tn916.

Keywords: Tn916, *S.pyogenes*, Transposable elements, Antibiotic resistance .

Introduction

*Streptococcus pyogenes* is the major human morbid connected with topical or systemic invasion and post-streptococcal immunologic disorders ¹. These bacteria colonize the throat or skin and cause several purulent infections ²,³. In addition, it may be stimulating autoimmune diseases ⁴. Increased levels of antibiotic resistance were reported in *S.pyogenes* in

many countries, particularly among groups includes (MLSB, β-lactams, Aminoglycosides, Tetracyclines and Sulfonamides) causing a problem of treatment failure in patients with these bacteria ⁵. In *S.pyogenes*, the prevalence of *tet*(M) may be dissected by this gene that is carried by “conjugative transposons” like Tn916 or by composite construction like Tn3701, which can in full swing translocate from chromosome to anther ⁶,⁷. Tn916 family, is a prototype of “conjugative transposons” widely dispersed in gram-positive streptococci, Tn916 was first detected in chromosomal DNA of *Enterococcus faecalis* strain DS16 ⁸. All transposable elements relationship to this family include the tetracycline resistance determinant *tet*(M), either solo such as Tn916 and Tn5397, or linked with another genes

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such as Tn6002, Tn6003, Tn3872, and Tn1545. Based on the above, to the importance of Tn916 in pervasion tetracycline resistance among bacterial, this study was strived to find the presence of transposable element in S. pyogenes.

Material and Methods:

Bacterial Isolates Identification:

Swab was collected from adults and children complain from upper respiratory infection Diyala, Baghdad and Erbil-Iraq, and Beirut Medical Center in Beirut-Lebanon for the time from January/2018 to April/2019. These samples were cultured on β-Selective Streptococcus agar medium (β-SSA), and the growing colonies were identified later by colonial morphology, gram staining, and biochemical tests (catalase test, blood hemolysis test, and bacitracin sensitivity test), furthermore bacterial isolates suspected to be S. pyogenes were identified by using Vitek-2 system and finally by molecular method.

Susceptibility testing:

Susceptibility of the versus Tetracycline, Minocycline, Doxycycline, and Oxytetracycline was checked, by virtue of to the disk diffusion by the Kirby-Bauer method.

Amplification experiments:

Amplification of tet(M) gene of Tn916 was performed by using specific primer

O6: 5’-GGTACTTGAAAGAACGGGAG-3’  and TETM11: 5’

TTCACCTTAGTATTTTCCACTG-3’

The dehydrated primer was solving in distilled water to reach a concentration of 10 picomole/μl. The amplification conditions began with primary and secondary denaturation at 95 °C, the first continued for 5 minutes, and the second continued for 30 seconds during which 30 cycles of denaturation were made. The other stage of amplification was the annealing process at 61 °C, which took only 30 seconds; the last stage was the initial and final extension process at 72 °C. The first took one minute, and the final one lasted for 7 minutes. Then PCR products were run by use electrophoreses technique.

Results and Discussion

Ninety-three bacterial isolates were obtained from clinical samples collected from pharyngitis, tonsillitis, and otitis cases from patients of different age groups and gender. All samples were cultured on β- selective Streptococcus agar and blood agar base supplemented with 5% of fresh human blood for determining the agricultural characteristics of colonies, then identified by using Vitek-2 system. Results of isolation showed that 93 bacterial isolate were characterized, among them 22 were identified as S. pyogenes. The prevalence of these isolates among clinical isolates is shown in figure (1).

Figure (1): Prevalence of S. pyogenes among clinical sources

Susceptibility of S. pyogenes isolates to tetracycline’s antibiotics was determined.

The results illustrated in figure (2) that resistance in the tetracycline group was 68.1%, 54.5%, 36.3% and 31.8% for minocycline, tetracycline, oxytetracycline and doxycycline, respectively. These resistance is consequent to the presence of tetracycline resistance gene tet(M) carried by Tn916, which mediates tetracycline resistance by the tetracycline-minocycline or tet(M) gene coding for tetracycline inactivating enzyme play a role in bacterial resistance to tetracycline, and that’s explains the tetracycline resistant phenotype in these isolates. However, one isolate symbolized H1 was sensitive to tetracycline, which may refer that this transposon harboring a silent copy of tet (M) as mentioned by Montanari et al., 13. Resistant isolates may presented tet (M) promoter that resulted in an increase
in the gene transcription thus causing a higher level of resistance as mentioned by El Moujaber et al., 14.

Figure (2): Percentage of tetracycline resistance among S.pyogenes isolates

International, many studies have indicated to rise in antibiotic resistance rate between S.pyogenes in tetracycline group 15. It seems clear that resistance significantly varies between geographical regions and time period variation, as well as, the increased use of antimicrobials, especially when misdiagnosing the disease, using unnecessary antimicrobials, or due to the irrational use of antimicrobials, as well as irregular consumption 12, 16.

Detection of Tn916

Tn916 is a conjugative transposon (or integrative conjugative element ICE) which was originally isolated from Enterococcus faecalis DS16 8. All transposable elements relationship to this family include the tetracycline resistance determinant tet(M), either solo such as Tn916 and Tn5397, or linked with another resistance genes such as Tn6002, Tn6003, Tn3872, and Tn1545 9.

In this study, Tn916 was detected in the clinical isolates of S.pyogenes by amplification of genomic DNA using specific primer (O6/TETM11), this primer targeting tet (M) gene carried by Tn916. Results explain in figure (3) appeared that there is an amplified product of 620bp shown the being Tn916 in eight isolates (36.3%) of S.pyogenes. All of these isolates are resistant to tetracycline. These findings are similar to those results detects Tn916 in S.pyogenes using the same primers 17. Resistant isolates may presented tet(M) promoter that resulted in an increase in the gene transcription thus causing a higher level of resistance 14. The results indicated that there was an isolation (symbolized H1) which was sensitive to tetracycline even though it contained a copy of Tn916 which indicates that this copy may be silent.

Moreover, results appeared that there are other eight isolates shown resistant for tetracycline, but they don’t have Tn916 which indicate that may be a chromosomal or plasmid copy of the tetracycline resistance gene, or cause by to another thematic tetracycline gene carried by other form.

Figure (3): Detection of Tn916 conferring tetracycline resistance

Prevalence of Tn916 among clinical isolates of S.pyogenes was illustrated in figure (4). Tn916 was prevalent in 7 out of 8 (87.5%) throat culture isolates and 1of 8 (12.5%) ear isolates. From these data, we conclude that Tn916 represents a specific element, which is more likely to occur in clinical S.pyogenes isolates.
Figure (4): Clinical sources of isolates and percentage of Tn916

Declarations:

Acknowledgement: We render our special thanks to all doctors and paramedical staff in Baqubeh Teaching Hospital for their help, time and openness during data collection.

Conflict of Interest: The authors declare that there is no conflict of interest.

Funding: The author (s) received no financial support for the research, authorship, and/or publication of this article.

Ethics approval and consent to participate:

Approval for the research was obtained after implementing the protocol recommended by the specialized committee in the Diyala Health Department and according to the official letter issued in 2018.

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Effect of Soft Cheese in Reducing of Lipid Profile and Liver Enzymes for Rats with Fat Disorder

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¹Post-Graduate, ²Associate Professor  Department of Food Science, College of Agriculture, Tikrit University, Tikrit, Iraq

Abstract

Aim: This study was designed to determine the impact of soft cheese upon total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), very low-density lipoprotein cholesterol (VLDL) and the efficacy of liver enzymes AST, ALT, and ALP in hyperlipidemia experimental Sprague-Dawley rats.

Materials & Methodology: Rats were divided into five groups (six rats each), healthy control group, hyperlipidemia control group, and three hyperlipidemia groups were fed on regular soft cheese (T1), cheese supplementation with probiotics (T2), and cheese supported with Nigella sativa L (T3). Following 28 days, Result: the results revealed a significant decrease (p<0.05) in total cholesterol in groups of rats that were feeding on chees T1, T2 and T3 (179.83, 138.71 and 135.06 mg/dl, respectively) compared to hyperlipidemia control group (250.98 mg/dl), T1, T2 and T3 groups showed a significant decrease in LDL levels (153.45, 104.39, 97.47 mg/dl respectively) compared to hyperlipidemia control groups (246 mg/dl). However, an increase of HDL levels were noticed in the same groups in contrast, T3 group revealed significantly decreased in the liver enzymes (28.45, 67.29, 98.16 IU/L) of ALT, AST and ALP respectively compared to hyperlipidemia control group (41.27, 88.35, 108.72)IU/L respectively.

Key words: soft cheese, probiotics, Nigella sativa L, lipid profile, liver enzymes, hyperlipidemia

Introduction

Cheese has known since prehistoric times, and it is believed, according to some ancient myths and recent archaeological discoveries, that the discovery of cheese occurred by chance by an Arab merchant who was transporting milk in containers made of sheep’s stomachs and crunches in the climatically warm Arab desert, which helped in the occurrence of milk cheese due to the effect of rumen and stomach enzymes, Then the cheese industry was moved from the Arab countries to Europe and various parts of the world¹. In the field of functional cheese production, it has produced many types of therapeutic benefits, as cheese is characterized by being a mild acid product and this accomplishes two purposes. The first is acceptance of acidic products and the second is increasing of the protection for improving micro-revitalization of health that is generated from fermented milk products such as yogurt. Therefore, the attention is drawn to cheese production that contains optimizing microbes². Medicinal plants are importance in many areas, including therapeutic, complementary, preventive and nutritional medicine. They contain many active constituents that are produced by the plant to accomplish many biological functions³. Among these medicinal plants is the Nigella sativa L., which is one of the most important medicinal plants used in direct medication or after extracting of the effective compounds for manufacturing drugs, and has been used to prolong the preservation of soft cheese⁴. Recent studies have pointed the role of probiotics, such as Bifidobacterium, Lactobacillus acidophilus, and Medicinal herbs, especially Nigella sativa L., which have a role in reducing heart disease risk⁵, The role of soft cheese fortified with black seed in reducing lipid profile and liver enzymes, and attributed the reason to that to effective compounds such as thymoquinone in black seed seeds⁶. That the consumption of Probiotic reduces blood fats due to the production of Hydroxy methyl-glutartete by lactic acid bacteria, which inhibits the enzyme glutaryl-CoA-Hydroxy methyl reductase⁷. This study aims to find out the effect of soft black bean-fortified functional cheese or probiotics on lipid profile and some liver enzymes in rats with hyperlipidemia.
Materials & Method

Probiotics: obtained from the Department of Food Sciences - College of Agriculture - the University of Baghdad in the form of a lyophilic in a tightly closed vial and included Lactobacillus acidophilus, Lactobacillus plantarum, and Bifidobacterium infantis.

*Nigella sativa L*: Nigeria Sativa seeds were obtained from the local markets of Samarra and their quality has been confirmed by the faculty members in the Field Crops Department.

Manufacture of cheese: The milk was divided into three groups. The pasteurization process was carried out on the three groups and the cheese was manufactured by following the steps mentioned 8. The following is done by pasteurizing the three groups with a temperature of 65°C for 30 minutes, after which the milk is cooled to a temperature of (45) °C and the thrombus was divided into three sections, the first without any treatment, and cheese was considered a control treatment (T1). As for the second part, it was added to it the black bean powder, and the treatment was considered (T2), and the third section was added to it with biological enhancers, and the third treatment was considered (T3) 9.

Experiment Animals: The experiment was conducted at the animals house of the College of Veterinary Medicine / Tikrit University, where 30 male rats (*Rattus norvegicus*) from the Sprague-Dawley strain at the age of 2-3 months and weights ranged between 205-215g. The experiment conditions were unified for all animals, where the room temperature was set between (23-25)°C by the use of an air conditioner, and the daily light period was 12 hours by the use of two fluorescent lamps, and the humidity rate was about 50%. Food and water were provided daily (*adlibitum*).

Standard meal preparation: The standard meal was prepared according to the 10 to contain (158.5 g casein / kg, 100 g glucose / kg, 50 g cellulose / kg, 100 G / corn oil / kg, 5 g multivitamin mixture / kg, 50 g mineral salts mixture / kg and 536.5 g grown / kg). Distilled water was added to the mixture to make a cohesive dough and to form the appropriate pieces to feed the rats, then placed in flat stainless steel utensils and dried in an oven at a temperature of 50°C through the hot air stream until the completion of drying and then packed in polyethylene bags and stored in the refrigerator when Temperature (2 ± 5) °C.

Preparing a high-calorie diet: The high-calorie diet was prepared using 11. the high-calorie diet of rats was prepared from previously prepared weighted food and then supplemented with fats (20% of hydrogenated fats and 5% of animal fats).

Design of experiment: The experiment animals were randomly divided into five groups, each group consisting of six animals. The animals were fed free for 28 days.

1-First group (negative control): these animals were left intact and fed on a standard diet only while continuing to give water for the duration of the experiment.

2- The second group (positive control): it was fed on a high-calorie diet (a fatty diet) for the duration of the experiment while giving distilled water of the experiment.

3- The third group (T1): It was fed on a fat diet in an amount 50% with soft cheese amount 50% for the duration of the experiment.

4- The Fourth Group (T2): It was fed on a fat diet in an amount 50% with soft cheese supported *Nigella sativa L* of amount 50% for the duration of the experiment.

5- The Fifth Group (T3): It was fed on a fat diet in an amount 50% with soft cheese supported probiotics amount 50% for the duration of the experiment.

Biochemical tests: After the end of the experiment period, the rats were prevented from food for approximately 12 hours Fasting, the animals were then drugged with chloroform, then blood was drawn directly from the heart and placed in test tubes that do not contain the substance (EDTA) and was left for about a quarter of an hour in a water bath at a temperature of 37 °C. Serum was then obtained by Centrifuge at 3000 rpm for 15 minutes and preserved at -20°C. until tests were conducted that included estimating total cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very low-density lipoproteins, enzyme Alanine transaminase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) has been estimated were determined in animal serums only according to the method 13.
Statistical Analysis: The results of the experiments were analyzed using the Linear Model General to study the effect of factors on the complete random design CRD and to determine the significance of the differences between the averages of the factors affecting the characteristics studied at the level of (p≤0.05).

Results and Discussion

The effect of functional soft cheese on the weight of the animal: Table (1) shows the effect of feeding a standard diet, a cholesterol-rich diet by 2%, a cholesterol-rich diet, with rats fed daily with 50% of the T1, T2, and T3 treatments, and a diet rich in cholesterol only, as the effect of different diets is observed on the daily and final weight increase between groups. Rats after 28 days of feeding showed that the daily weight of the rat's group that fed on a standard diet was reached to 0.133 g/day, while the final weight was increased to 3.74 g.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bodyweight (g)</th>
<th>Bodyweight gain</th>
<th>Average daily increase in body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial body weight</td>
<td>Final body weight</td>
<td></td>
</tr>
<tr>
<td>negative control</td>
<td>214.35 ±b3.21</td>
<td>218.09 ±d1.28</td>
<td>3.74</td>
</tr>
<tr>
<td>positive control</td>
<td>254.67 ±a4.53</td>
<td>277.50 ±a2.75</td>
<td>22.83</td>
</tr>
<tr>
<td>T1</td>
<td>252.81 ±a2.15</td>
<td>260.34 ±b2.37</td>
<td>7.53</td>
</tr>
<tr>
<td>T2</td>
<td>255.16 ±a3.22</td>
<td>263.64 ±b2.29</td>
<td>8.48</td>
</tr>
<tr>
<td>T3</td>
<td>254.44 ±a3.58</td>
<td>250.32 ±c2.08</td>
<td>-4.12</td>
</tr>
</tbody>
</table>

The numbers in the table express the mean values of the mean standard deviation. Different letters in one column indicate significant differences (p <0.05) between study groups.

The results show that the highest daily increase has appeared in rats feed on a diet rich in cholesterol, with an average daily increase of (0.815)g/day and the final weight increase is (22.83)g, while the average daily weight increase in the members of the rat’s group fed a diet rich in cholesterol and fed by treatment T1 reached (0.268) g/day and the final increase is (7.53) g while in the rats fed a rich diet cholesterol and fed by treatment T2 (0.302)g/day and the final weight increase is (8.48) g/day. The weight increase in the average weight of animals consuming diets rich in cholesterol is the result of the high concentration of both cholesterol and triglycerides as well as the increase in low-density fats. This increase is normal as a result of animal feeding on cholesterol. The increase in treatment T1 is due to the influence of cheese proteins and this is consistent with what found, which indicates that cheese proteins are an important source of amino acids that are of great importance in building protein in the body, in particular the amino acid leucine. It also leads to more muscle adaptation to exercise and contraction for the purpose of bodybuilding. The increase in treatment T2 was due
to the black bean containing carbohydrates, fats, and proteins. The reason for the lower weight of the rats in treatment T3 is the degradation of subcutaneous fat tissue due to the feeding of the rats with vital boosters.

The effect of functional soft cheese on the lipid profile parameters: Table (2) shows the occurrence of significant changes (P ≤ 0.05) in biochemical tests of experimental groups. The total cholesterol level in the affected animal group increased to (250.98) mg/dl compared to that in the healthy control group (108.11) mg/dl, while the treatment with T1, T2, and T3 showed a significant decrease in the cholesterol level (179.83, 138.71 and 135.06) mg/dl, respectively. The results of the biochemical analysis also showed a significant decrease in the level of probability (P ≤ 0.05) in the level of high-density lipoproteins for the affected group to reach (44.16) mg/dl compared to the healthy control group that reached (59.0) mg/dl. While it was observed that HDL-C in groups of infected animals treated with T1, T2, and T3 treatments increased significantly to (52.56, 55.39 and 57.20) mg/dl, respectively. Also, a significant increase in the level of low-density lipoproteins is observed for the group of infected animals, which amounted to (246) mg/dl compared to the healthy control group which reached (64.71) mg/dl. While a significant decrease was observed for the level of LDL-C in the blood serum of animals fed by the three treatments to record (153.45, 104.39, and 97.47) mg/dl, respectively.

This increase may be due to an increase in the cholesterol acyl transferase enzyme activity, which is responsible for absorbing cholesterol that stimulates when insulin deficiency is caused by oxidative stress that affects pancreatic beta cells by the effect of the active oxygen classes, thus increasing the level of cholesterol absorption from before the intestine. The results also showed a significant decrease in the total cholesterol

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative control</td>
<td>108.11 ±d1.29</td>
<td>78.01 ±e1.28</td>
<td>59.00 ±a1.17</td>
<td>64.71 ±e0.97</td>
<td>15.60 ±d0.09</td>
</tr>
<tr>
<td>positive control</td>
<td>250.98 ±a1.57</td>
<td>195.91 ±a1.35</td>
<td>44.16 ±d0.83</td>
<td>246.00 ±a4.28</td>
<td>39.18 ±a1.68</td>
</tr>
<tr>
<td>T1</td>
<td>179.83 ±b2.41</td>
<td>130.91 ±b0.89</td>
<td>52.56 ±c2.43</td>
<td>153.45 ±b1.85</td>
<td>26.18 ±b0.15</td>
</tr>
<tr>
<td>T2</td>
<td>138.71 ±c3.04</td>
<td>105.37 ±c1.15</td>
<td>55.39 ±bc0.89</td>
<td>104.39 ±c1.13</td>
<td>21.07 ±c0.88</td>
</tr>
<tr>
<td>T3</td>
<td>135.06 ±c2.19</td>
<td>98.05 ±d2.63</td>
<td>57.20 ±b1.63</td>
<td>97.47 ±d1.36</td>
<td>19.61 ±c0.79</td>
</tr>
</tbody>
</table>

The numbers in the table express the mean values of the mean standard deviation.

Different letters in one column indicate significant differences (p < 0.05) between study groups.
concentration in the serum of T2 group rats, and these results are consistent with the results of many studies, which indicate that the black seed can be effective in reducing the level of lipid profile. This may be because the essential oils of black seeds that have a great anti-oxidant activity for being contain thymoquinone compound, which is one of the sweeping factors of the free hydroxyl root, preventing lipid peroxidation. These results are consistent with what AL-Jobouri found that the consumption of foods fortified with Probiotic in the form of pharmaceutical products or in the form of ferments for a container structure on the bacteria Lactobacillus and Bifidobacterium, which are among the most important foods that contribute to reducing the level of lipid profile as the boosters work. It is vital to lower cholesterol and thus reduce chronic heart disease. Shown also found that the production of Hydroxymethylglutarate by probiotics inhibits the reductase glutaryl-CoA-Hydroxymethyl enzyme, which is a requirement in the process of cholesterol formation and reduces heart disease rate 2-3%. This is also consistent with what Aktimur et al., mentioned that Probiotic bacteria work to reduce the level of cholesterol in the blood, although this decrease may be affected by other factors such as the bacterial strain or the supports used and others, and the reason for the decrease in blood fat may be that these Biologists produce enzymes that break down fat and some of them destroy cholesterol to make use of it as a carbon source.

The effect of functional soft cheese on some enzymatic parameters: Table (3) shows that ALT level showed the highest concentration in the serum of the infected control rats group (41.27) IU/L, while its concentration in the affected and fed rats group on the T1, T2 and T3 (35.89, 31.19 and 28.45) IU/L, respectively, and compared to the healthy rat’s group, which amounted to (24.71) IU/L. As for the enzyme AST, it was the highest concentration in the serum of the affected control group as it reached (88.35) IU/L and compared to the healthy control group that recorded (63.48) IU/L and it is showing that there are significant differences at the probability level (P≤0.05), either When adding the T1, T2, and T3 coefficients, their value decreased to (76.95, 70.34 and 67.29) IU / L, respectively,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative control</td>
<td>24.71 ±0.85</td>
<td>63.48 ±2.05</td>
<td>94.53 ±1.56</td>
</tr>
<tr>
<td>positive control</td>
<td>41.27 ±1.14</td>
<td>88.35 ±1.27</td>
<td>108.72 ±2.02</td>
</tr>
<tr>
<td>T1</td>
<td>35.89 ±1.03</td>
<td>76.95 ±1.76</td>
<td>103.44 ±2.25</td>
</tr>
<tr>
<td>T2</td>
<td>31.19 ±0.95</td>
<td>70.34 ±1.85</td>
<td>98.39 ±0.57</td>
</tr>
<tr>
<td>T3</td>
<td>28.45 ±0.88</td>
<td>67.29 ±1.33</td>
<td>98.16 ±1.30</td>
</tr>
</tbody>
</table>

The numbers in the table express the mean values of the mean standard deviation.
Different letters in one column indicate significant differences (p <0.05) between study groups.

Compared to the affected control group. The highest value of ALP was recorded in the serum of the affected control group as it reached (108.72) IU/L. while its concentration in T1, T2, and T3 coefficients (103.44, 98.39 and 98.16) IU/L, respectively. The level of the enzyme AST and ALT increases during infection with viral hepatitis and other liver diseases accompanied by the occurrence of necrosis in the cells of the liver, as the level of the enzyme increases before the emergence of shows pathological symptoms to the equivalent of (10-100) once more than its normal rate and also increases its activity showily in some Conditions such as acute hepatitis 24. The level of the ALP rises in a variety of conditions, including Cholestasis, Infiltrative liver disease, Partial and Total blockage of the bile duct, Pregnancy, Bone regeneration, Granuloma of the liver and Neoplastic liver disease, on the other hand, the increase in the concentration of basal phosphatase may It is because of the increased efficacy of Lysosomes, which is one of the important pre-cellular changes 25. The results of group T2 indicate a decrease in the concentration of enzymes in the serum of the experiment rats, and the results of the study are consistent with what Mahmoud et al, 26 observed. There was a significant decrease in the concentration of enzymes (AST, ALT) in the serum of the rats fed on soft cheese supported by black seeds. and also with the results Al-Seeni et al, 27, there was a significant decrease in the concentration of liver enzymes in serum rats with hypercholesterolemia and fed on black seed oil. It is also evident from the results that the T3 group had a low enzyme concentration as well due to the decrease in the role of Lactobacillus and Bifidobacterium in improving the metabolic indicators in the liver and then improving its functions through improving the metabolic processes are consistent with what Javadi et al, 28.

Conclusion

Feeding with high-fat diet affected the blood lipid profile if it increased it, while treatment with both functional soft cheese fortified with black seed and probiotics led to a decrease in these indicators. Regarding liver enzymes, feeding on a high-fat diet led to an increase in it, while treatment with functional soft cheese reduced the level of liver enzymes.

Ethical Clearance: Ethical clearance from the institutional ethical committee obtained for the study.

Conflict of Interest: Nil

Source of Funding: Self funding.

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Investigation of Lumpy Skin Disease Virus in Baghdad City

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Abstract

In this study, the lumpy skin disease virus was isolated on the lamb testis cell culture for the first time in Iraq. Forty skin nodules were collected from cows suspected with lumpy skin disease (LSD) in Baghdad governorate. Real-time PCR detected LSD virus in 100% of these skin nodules. After that, the lamb testis cell culture was prepared for virus isolation. The prepared samples (0.5 ml) were inoculated into prepared lamb testis cell culture. Cytopathic effects (CPE) of the virus have appeared after 24hr post-inoculation and completed within 72hr. The noticed CPE were cell rounding, aggregation of cells, syncytia formation, and detached of the cells from the cell sheet. Fluids of lamb testis cell culture were examined by the real-time PCR to confirm that the observed CPE was due to the LSD virus. Cell culture fluids were positive by the real-time PCR (100%). Real-time PCR was sensitive for the detection of the LSD virus DNA in skin nodules, and it was a suitable confirmatory diagnosis tool of the virus in the tissue culture fluid. Finally, the locally isolated LSD virus in this study is valuable in future studies for making a vaccine to control the disease.

Keywords: Cattle, Cell culture, Lumpy skin disease, Real-time PCR, Skin nodules

Introduction

Lumpy skin disease (LSD) is a cattle pox illness caused by a virus called the Neethling virus. It mostly affects cattle and zebus but was also seen in giraffes and impalas¹. The disease is characterized by fever, nodules on the skin, internal organs and mucous membranes, swollen lymph nodes, emaciation, skin edema, and occasionally death². Abortion and pneumonia are the most usual sequels to LSD whereas the latter may be potentially fatal³. Lumpy skin disease virus (LSDV) has a double-stranded DNA genome that replicates in host cells cytoplasm⁴. This virus belongs to the Poxviridae family, of the Chordopoxvirinae subfamily, in the Capripoxvirus genus⁵. LSD is the main cattle health issue causing significant economic losses due to decreased milk production, a prolonged weakening of the clinical course, weight gain reduced, sterility in bulls, abortion of pregnant cows and permanent skin damage has a significant effect on the leather industry and this leads to a ban on international livestock trade⁴. Due to its economic effects on the global cattle industry, the World Organization of Animal Health (OIE) has listed the LSD virus as a notifiable disease¹. OIE reported that the latest outbreaks of LSD when to occur in Iraq and Turkey, extending concerns that the disease will persist for spread into Asia and Europe²³.

In Iraq, LSD has occurred since 2013, and LSDV is circulating between Iraqi cows⁶. In autumn 2014, LSDV was detected by the polymerase chain reaction in Babil, Al-Qadysia, and Al-Muthana Governorate⁷, was reported the occurrence of Lumpy skin disease among Iraqi cattle in Wasit province, ⁹, was recorded the LSD in Al-Qadysia province. There are many studies in Iraq were conducted about the detection of LSD virus¹⁰, but the virus did not isolate so it was important to isolate LSD virus on cell culture to aid in the manufacture of a vaccine to control this disease in the future.

The aim of this study was the isolation of lumpy skin disease virus for the first time in Iraq on cell culture then the isolation will confirm by molecular technique.
Materials and Methods

Samples collection

Throughout July to October 2019, forty (40) skin lesions were collected in sterile containers from animals that showed nodules or sitfasts on all the entire body, enlargement of lymph nodes, excessive salivation, mucous membrane ulceration, milk production decrease, anorexia, and pneumonia. These samples (sitfasts and nodules) were collected from five areas in Baghdad city (Abo-Ghraib, Al-Kadhimiya, Al-Wahda, Al-Ridhwania, and Al-Husua).

Extraction and amplification of DNA from collected samples

The collected samples divided into two parts, one part for detection of LSD virus by the real-time PCR, and the other for isolation of the virus on lamb testis cell culture. After sample preparation, the DNA was extracted by an extraction kit (Qiagen, Germany). Then, the extracted DNA was amplified by the real-time PCR kit (Genekam Biotechnology AG, Germany).

Preparation of primary lamb testis cell culture

Lamb testis cell culture Preparation was conducted in the virology laboratory of Baghdad University according to11.

Isolation of LSD virus on lamb testis cell culture

After four days, the cell monolayer growth was completed, and growth media was removed from all flasks. LSD virus was propagated in lamb testis (LT) cell culture by inoculation 0.5 ml of the prepared sample into all flasks (except control flasks). Then, flasks were incubated at 37°C for virus adsorption (1 hour). Thereafter, media (maintenance) was added to all flasks (infected and control), incubated at 37°C, and cells were monitored daily by an inverted microscope for cytopathic effects. After that, flasks were frozen at -20°C (first passage). Flasks of the first passage were thawed to repeat the inoculation of undiluted fluid on new cells (second passage).

Detection of isolated LSD virus in cell culture by real-time PCR

After thawing of all infected flasks (passage 1 and 2), 0.5 ml was taken from each one of these flasks and were subjected to the extraction and amplification of DNA by real-time PCR for confirmation of the isolation of LSD virus in cell culture.

Results and Discussion

Clinical investigation of lumpy skin disease

Animals that used in this study were showed firm, circumscribed, raised and rounded nodules or sitfasts on all the entire body (figure 1), excessive salivation and nasal discharge, mucous membrane ulceration (figure 2), enlargement of lymph nodes, decrease in milk production, anorexia, and pneumonia. These results were compatible with12, 13 and 14.

Detection of LSD virus in the collected samples by using real-time PCR technique

In the current study, skin nodules collected from naturally infected cattle detected by real-time PCR (Figure 3) and were positive (100%). These results revealed that the real-time PCR was sensitive in the detection of LSD virus in skin nodules of naturally infected cattle and indicated the incidence of an outbreak
of lumpy skin disease in Baghdad governorate in 2019. The findings of this study are consistent with those obtained by other researchers who reported that the percentage of positive skin samples was 100%. However, these results are not compatible with, who pointed the detection of the LSD virus in 72% of skin lesions which is probably because of the collection of some samples were in the convalescent stage or due to presence of other diseases that clinically confused with LSD such as pseudo-lumpy skin disease.

![Figure 3: The plot of real-time PCR amplification of positive samples (L406, L403, L401, L408, L410, and L409) from Al-Husua, giving a Ct value of 27, 28, 29, 30, 31, and 33 respectively. +ve: positive control give a Ct value of 28.-ve: negative control (did not give a Ct value).](image)

**Isolation of LSD virus in lamb testis cell culture for the first passage**

The cytopathic effects of the LSD virus were rounding and aggregation of cells noticed after 24 hours post-inoculation. After 48 hours, the number of rounded cells increased, some of them separated from the flask surface, and the cells lost their cellular borders. Within 72 hours post-inoculation, the cytopathic effects were complete by observing the cell clusters detached from the cell sheet leaving empty spots and syncytia formation (Figures 4).

![Figure 4: Cytopathic effects of LSD virus after 72hr post-inoculation (first passage), show syncytia ( ) (100x). Sample no. L379 from Al-Ridhwania.](image)
Isolation of LSD virus in lamb testis cell culture for the second passage

The cytopathic effects of lumpy skin disease virus on lamb testis cell culture in the second passage (Figures 5 and 6) were fast, diffuse, and more evident than the first passage. The type of cytopathic effects was similar (as described in the first passage). In the present study, the primary lamb testis cell cultures were suitable for the cultivation of the virus, and the CPE appeared after 24hr post-inoculation in the first passage while in the second passage the CPE appeared in less than 24hr post-infection. This is similar to the studies of \cite{18} and \cite{22}. However, these findings are contrary to that published by \cite{17} and \cite{19}, who pointed out that the CPE appeared within three days. The CPE of LSD was characterized by cell rounding, cell aggregation, syncytia formation, and detached of the cells from the cell sheet. These findings corresponded with the described results of other authors \cite{17, 18, 19} and \cite{21}. In this study, the cytopathic effects were completed within 72hr which found to coincided with \cite{22} who recorded that the CPE completed in 48 to 72hr but it does not agree with \cite{20} who reported that CPE completed in 9 days. These variations in the time of CPE appearance and completion were probably related to the type of cell culture, the dose of inoculated virus, strain of virus, media, and serum used.

![Figure 5](image5.png)

**Figure 5:** Cytopathic effects of LSD virus after 72hr post-inoculation (second passage), showed cell rounding, aggregation of cells, and empty spots (200x). Sample no. L379 from Al-Ridhwania.

![Figure 6](image6.png)

**Figure 6:** Cytopathic effects of LSD virus after 72hr post-inoculation (second passage), showed rounding of all cells (400x). Sample no. L399 from Al-Kadhimiya.

Detection of isolated LSD virus in cell culture by the real-time PCR technique

All fluids of cell culture (from both passages) were examined by the real-time PCR were positive (100%). This confirmed that the cytopathic effects were due to the LSD virus.

Conclusions

Incidence of infection with LSD virus in Baghdad governorate in 2019 was documented. The real-time PCR was sensitive for the detection of the LSD virus in skin nodules, and it was a suitable confirmatory diagnosis tool of the virus in the tissue culture fluid. It was favorable to collect skin nodules for diagnosis and isolation of the LSD virus. The primary lamb testis cell culture was suitable for lumpy skin disease virus isolation.

Ethical Clearance – This research was carried out under an agreement with the guidelines of the Canadian Council regarding using lab animals and animal care [Olfert] and approved by the office of the agricultural research ethics committee that recently took place in Iraq (approval number 5849/AGRO).

**Source of Funding** – Self

**Conflict of Interest** - Nil

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Laboratory Evaluation of the Entomopathogenic Fungi
*Penicillium Marneffei* and *Verticillium lecanii* Against *Culex Pipeins* Moletus

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Abstract

**Background:** The biological efficacy of *Penicillium marneffei*, *Verticillium lecanii* in the biological control of the second and third larval phases and adult male and female mosquitoes was studied. *Culex pipeins molestus*. **Aim of the study:** This research aimed to isolate *P. marneffei* and *V. lecanii* associated with *C. pipeines* mosquitoes and compare the biological efficiency of *P. marneffei* and *V. lecanii* in their biological control second and third instar and adult for the *Cx.pipeins*. **Materials and Methods:** study was conducted for a period of 3 months from March 2020 to May 2020. **Results:** killing increased with increasing concentration due to an increase in the number of developing spores. The results of the laboratory tests are shown *V. lecanii* fungi. at concentration $2 \times 6^7$ spore / ml highest mortality ratio 89.63% for the second larval age after 72 hours, the killing rate was 81.59% for males and 92.67% for females after 72 hours when using $2 \times 6^7$ spore / ml. **Conclusion:** Entomopathogenic Fungi *P. marneffei* and *V. lecanii* can use secondary metabolites in the manufacture of a fungicide for the purpose of controlling *Cx.pipeins* mosquito.

**Keywords:** *Penicillium marneffei*, *Verticillium lecanii*, *Culex pipeins*

Introduction

Agricultural pest control using fungal pathogens formed during Last five-fifth years fertile ground for scientific research, it has been defined More than 700 species of pest pathogenic ¹. The pest pathogen is undoubtedly an important biological enemy In general, the pathogenic forms reach the host and then spread and penetrate his body, depending on their stock food. Whereas, these spores grow within the host in succession². The form of monocytes or multicellular cells (hence, bud spores Protoplast), taking advantage of the food sources of the overworked host². The pathogen develops and dies, while the fungus results in a number. The infection secures the spread of the fungus A large source of spores Pathogen, or when the fungus resorts to produce a resistant fungal structure that allows for prolongation How long it is without host ³. Insect pathogenic fungi are one of the most common and most distinctive pathogens of insect diseases fungi produce spores that stick to the insect’s body and then germinate when the conditions are right. Therefore, send a tube that produces enzymes that release enzymes at the point of contact with the cuticle of the insect, analyzing the proteins, chitin and lipids involved in the synthesis of cuticle. *P. marneffei* and *V. lecanii* are fungi associated with insects, and are considered insect pathogenic fungi due to their widespread presence in nature ⁴. There are about 35 species of mosquitoes in nature. *Culex pipeins* is one of the most common species in the central and southern regions of Iraq. ⁵ Misleading sites of plants and weeds are more attractive to female mosquitoes ⁶. Eggs from exposed sites, mosquitoes *Cx. pipeines*, one of the most common mosquito species in the world, is a biological vector for many pathogens of humans and animals, including the transmission of filariasis parasite ⁶,⁷. Bio-control using some fungi is currently one of the best ways to control mosquitoes. Through the use of spores containing *Cx.pipeins* ⁸.

**Aims and Objective**

This research aimed to isolate *P. marneffei* and
\textit{V. lacanil} associated with \textit{C. pipeines} mosquitoes and compare the biological efficiency of \textit{P. marneffei} and \textit{V. lecenii} in their biological control second and third instar and adult for the \textit{Cx.pipeins}.

### Materials and Methods

#### Fungi Isolation

\textit{Cx. pipeines} larvae were sterilized by ethyl alcohol 70% For one minute sodium hypochlorite was used for 30 seconds for sterilization and placed on filter paper for disposal of sterile residue. Four replicates were prepared from Potate dextrose agar medium, and five larvae were placed in each repeater. By sterile forceps, the dishes were incubated in a Syrian-made Jard incubator at a temperature of 25 ± 2°C for seven days, a 0.4 cm tablet was taken from the edge of the growing colony around the larvae and placed in a petri dish containing 20 ml of the above food medium and used for this purpose a sterile needle Incubate at 28 ± 2°C for seven days. Multiple ponds were selected at sites in Salheldin province where \textit{C. pipeins} in these ponds because they are rich in organic materials, were collected from different sites for each pond during the march to May 2020 then transferred to the laboratory and emptied in plastic ponds, and the larval food of ground mice, consisting of corn, wheat and protein was added by 1:1:1 by 2 g per basin.

Insect breeding was carried out in the laboratory at a temperature of 25 ± 2°C relative humidity 65 ± 5 light a period of 12 hours / day. \textit{P. Marneffi} and \textit{V. lecenii} isolated from \textit{Cx.pipeins} larvae were diagnosed by fungal growth and caterpillar color by taking a small portion of the fungal growth and placing it on a glass slide with a drop of lcto phenol cotton blue and then cover the slide and examined under a microscope under 40x magnification.

#### Preparing commenter fungi

Glass flakes 250 ml were used for the preparation of the suspension for \textit{P. marneffi} and \textit{V. lecenni} by taking a 0.5 cm diameter tablet from the fungal colony growing on P.D.A. The tablet was placed in 9.5 ml sterile distilled water in the jug above and shaken for five minutes to remove the spores from its spore mounts. Culture was incubated at 25 °C for 7 days, and for the purpose of distributing the fungal growth was shaken daily, a piece of paper was used for filtration and get commenter fugi using a modified erythrocyte slice count to calculate the number of Haemocytometer spores. Following the concentration:

- $2 \times 10^5$ spore / ml
- $2 \times 10^6$ spore / ml
- $2 \times 10^7$ spore / ml

Bioassay in larval stages of \textit{Cx.pipeines} molestes

Four containers/ type of fungus were used, each containing 20 larvae per instars II and III and each concentration of \textit{P. marneffei} and \textit{V. lecenii} tested by using 50 ml distilled water was used to control. The larvae were test left in the plastic pots for 2 minutes. The larvae were then transported by a soft brush to glass jars of 200 ml each distilled water container and the larval food at 10 mg / ml and placed in the incubator at 28 ± 2°C and a light period of 72 hours, the mortality rate was calculated after 48 hours and 72 hours.

Bioassay in adult mosquitoes, \textit{Cx.pipeins} molestes

Numbers of pupae were placed individually in 20 mL tubes and cotton was used for the purpose of closing the nozzle of the tube and waited to become adults. 10 adult males and females were each distributed separately in a 1-liter wide-mouthed pot with three replicates per concentration. In addition to the control Factor, each repeater was sprayed by a fungus at a distance of 5 cm, while the control factor was sprayed with water and placed in the incubator at a temperature of 2±.28 °C and a light period of 12 hours, after which the mortality rate was calculated after 48, 72 hours.

### Statistical Analysis

The results were analyzed based on multiple comparisons between the coefficient rates of the experiment using the complete random design (CRD). The results were analyzed using Duncan Multiple Range Test to find the differences between coefficients according to the significant differences between them and at the level of significance specified for the test (P < 0.05).

### Results and Discussion

The results of the laboratory tests are shown in the table. The fungus \textit{P. marneffei} at concentration 2 ×
6^7 spore /ml gave highest mortality ratio 83.92% for the second larval age after 72 hours while the fungus *P. marneffei*. at concentration 2 × 6^7 spore /ml highest mortality ratio 68.83% for the third larval age after 72 hours of treatment the rate was 0% in the control coefficient, and the relationship between concentration and rate was direct and that this relationship was clear between the duration of exposure and rate. For larval phases II and III where as the proportion of killing increased with increasing concentration due to an increase in the number of developing spores as well as the impact of the immune system, which can not defend the body at high concentrations of the fungus\textsuperscript{11}.

The statistical analysis shows that there are significant differences in the killing rate for the phases at the level of 0.05 and the killing rates decrease with increasing larval age and when exposing the second and third larval phases. The reason is that the immune system of the second larval instar is incomplete and the insect body will be thin.

Table (1) shows larval stages of *Cx. pipeins molestes* affected by various concentrations.

<table>
<thead>
<tr>
<th>Type of fungi <em>P. marneffei</em></th>
<th>Phases</th>
<th>Concentration Spore / ml</th>
<th>Percentage of mortality</th>
<th>Average concentration effect</th>
<th>Average treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2×10^5</td>
<td>34.52</td>
<td>73.41</td>
<td>53.83 C</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>2 x 106</td>
<td>43.74</td>
<td>78.68</td>
<td>61.21 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 107</td>
<td>63.58</td>
<td>83.92</td>
<td>73.74 A</td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td>2x10^5</td>
<td>23.89</td>
<td>56.83</td>
<td>40.36 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 106</td>
<td>28.65</td>
<td>62.69</td>
<td>45.67 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 107</td>
<td>45.62</td>
<td>68.83</td>
<td>57.22 C</td>
</tr>
<tr>
<td></td>
<td>Control</td>
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<td>0</td>
<td>0 E</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0 C</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>Average time effect</td>
<td>47.28 b</td>
<td>78.66 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td></td>
<td>32.72 b</td>
<td>62.78 a</td>
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</tr>
</tbody>
</table>

* Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.
**Bioassay of fungi suspension in adult mosquitoes.**

*Cx.pipeins molestue*

The results of the laboratory tests are shown in the table(2). *P. marneffei*. Against mosquito adults, with the highest homicide Cx. *pipeins molestue* rate was 79.17% for males and 89.42% for females after 72 hours when using $2 \times 10^7$ spore. The mechanism of action of the fungus against male and female adults is the penetration of the thin areas of cuticle after adult spraying with the fungus; the fungus grows and the problem of filamentous growth is multiplied between the tissues of the body of the insect then send the fungus carriers outward and then the death of the insect 12.

**Table (2). Effect of different concentrations of fungi suspensions in the P. marneffei destruction of adult males and female mosquitoes Cx. pipeines molestues.**

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>Adult</th>
<th>Concentration</th>
<th>Percentage of adult mortality</th>
<th>Average concentration effect</th>
<th>Average treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spore / ml</td>
<td>24 h</td>
<td>72 h</td>
<td></td>
</tr>
<tr>
<td>P. marneffei</td>
<td>Male</td>
<td>$2 \times 10^5$</td>
<td>45.94</td>
<td>67.41</td>
<td>56.67 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^6$</td>
<td>56.95</td>
<td>73.83</td>
<td>65.39 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^7$</td>
<td>67.72</td>
<td>79.17</td>
<td>73.17 B</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>$2 \times 10^5$</td>
<td>53.72</td>
<td>65.96</td>
<td>59.84 D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^6$</td>
<td>68.21</td>
<td>84.36</td>
<td>76.28 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^7$</td>
<td>78.18</td>
<td>89.42</td>
<td>A 83.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Average time effect</td>
<td>62.50 b</td>
<td>78.71a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Average time effect</td>
<td>66.70 b</td>
<td>79.91 b</td>
<td></td>
</tr>
</tbody>
</table>

* Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.
The results of the laboratory tests are shown in the table(3) V. lecanii fungi at concentration $2 \times 10^7$ spore / ml highest mortality ratio 89.63% for the second larval age after 72 hours while giving to the fungus V. lecanii at concentration $2 \times 10^7$ spore/ml highest mortality ratio 72.23% for the third larval age after 72 hours of treatment while the rate was 0% in the control coefficient, and the relationship between concentration and rate was direct and that this relationship was clear between the duration of exposure and mortality rate for larval phases II and III, whereas the proportion of killing increased with increasing concentration and exposure period in killing rates increased concentration is due to an increase in the number of developing spores as well as the impact of the immune system, which can defend the body at high concentrations of the fungus Its efficiency $^{13}$. The statistical analysis shows that there are significant differences in the killing rate for the phases at the level of 0.05 and the killing rates decrease with increasing larval age and when exposing the second and third instar.

The reason is that the immune system of the second larval phases is incomplete and the insect body will be thin which is porous by the fungi spores. The findings were consistent with $^{14}$. Describe the relationship between the concentration of spores and the percentage of killing as a direct relationship, where the greater the concentration rate, the greater the proportion of killing This laboratory study converged with the study $^{15}$. Which proved that the biological control using the fungus *Aspergillus niger* at the concentration of $1 \times 10^4$ spore /ml for the larvae of different phases of mosquitoes.

* *Cx..quinquefasciatus* gave a killing rate of 87.68%. Murder The results of the study were similar with $^{16}$ a 90% killing rate when exposure of *Cx.quinquefasciatus* mosquito larvae to *Metarrhizium anisopliae* spores at $2 \times 10^6$ spore / ml. This study is consistent with a study $^{17}$ that demonstrated that the use of different concentrations of fungus

* *Metarhizium brunneum* resulted in 95% killing of the first larval stage of *Cx.pipeins*.

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>Phase</th>
<th>Concentration Spore / ml</th>
<th>Percentage of adult mortality</th>
<th>Average concentration effect 24 h</th>
<th>Average concentration effect 72 h</th>
<th>Average concentration effect 72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. lecanii</td>
<td>Second</td>
<td>$2 \times 10^5$</td>
<td>49.73</td>
<td>56.52</td>
<td>53.12 D</td>
<td>71.91 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^6$</td>
<td>76.62</td>
<td>79.21</td>
<td>77.91 B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^7$</td>
<td>81.26</td>
<td>89.63</td>
<td>85.44 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td>$2 \times 10^5$</td>
<td>38.18</td>
<td>67.53</td>
<td>52.85 D</td>
<td>58.32 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^6$</td>
<td>49.42</td>
<td>55.18</td>
<td>52.33 D</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^7$</td>
<td>67.43</td>
<td>72.32</td>
<td>69.87 C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>Average time effect</td>
<td>63.20 b</td>
<td>75.12 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td>Average time effect</td>
<td>51.67 b</td>
<td>65.02 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.*
Table (4) shows the effect of different concentrations of the \textit{V. lecanii} in mosquitoes. The killing rate was 81.59\% for males and 92.67\% for females after 72 hours when using $2 \times 6^7$ spore / ml concentration. The mechanism of action of the fungus against male and female adults is the penetration of the thin areas of the cuticle after adults spray the fungus, the fungus grows and the problem of filamentous growth between the tissues of the insect’s body subsequently increases. The fungus sends conidic carriers outward and then the death of the \textsuperscript{18} Current results show similarity with some previous research, where the use of \textit{V.lecanii} against females \textit{Cx. pipiens} resulted in a 100\% killing rate within four days and when the use of \textit{V.lecanii} against mosquito adults \textit{Cx}. 93\% at $2 \times 5^{10}$ spore / ml concentration after 168 hours while females killed 96\% at the same concentration and duration \textsuperscript{19}. Males lost 93.33\% and females 90\% after 168 hours.

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>Adult</th>
<th>Concentration spore / ml</th>
<th>Percentage of adult mortality</th>
<th>Average concentration effect</th>
<th>Average concentration effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{V. lecanii}</td>
<td>Male</td>
<td>2x105</td>
<td>34.73</td>
<td>48 h 68.51</td>
<td>61.29 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 106</td>
<td>42.32</td>
<td>74 h 71.94</td>
<td>57.13 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 107</td>
<td>68.67</td>
<td>81 h 81.59</td>
<td>75.13 A</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2x105</td>
<td>51.35</td>
<td>48 h 73.16</td>
<td>62.25 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 106</td>
<td>67.56</td>
<td>74 h 83.37</td>
<td>75.46 B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x 107</td>
<td>68.31</td>
<td>92 h 92.76</td>
<td>89.03 A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>D 0</td>
</tr>
<tr>
<td>Male</td>
<td>Average time effect</td>
<td>48.57 b</td>
<td>74.01 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Average time effect</td>
<td>62.07 b</td>
<td>71.11 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.

The \textit{P. marneffei} and \textit{V. lecanii} fungus infects mosquito larvae naturally in aquatic environment. Use an insect pathogenic fungus to reduce its spread about pesticide within integrated management programs are an important alternative chemicals that, in many areas, cause the disturbance of the vital balance they led to the emergence of secondary pests as major pests as a result of damaging their vital enemies prevalent in these areas. Especially as many of these pathogenic fungus it is naturally present in many of our arab environments to return pioneering role in limiting the spread of many of the pests throughout our homeland. The study proved that entomopathogenic fungi \textit{P. marneffei} and \textit{V. lecanii} have a significant effect on the life second, third instar and adult of \textit{Cx.pipiens}. It can be introduced into the pest management program using secondary metabolites
in the manufacture of a fungicide for the purpose of controlling Cx.pipiens mosquito.

**Conclusion**

The results of the laboratory tests are shown *V.lecanii* fungi at concentration $2 \times 6^7$ spore / ml highest mortality ratio after 72 hours The study proved that the insect entomopathogenic *P. marneffei* fungi. *V.lecanii* has a significant effect on the life of the larvae of the second and third instar, as well as the adult male and female mosquitoes Cx.pipines, so that a fungicide can be manufactured for the purpose of controlling mosquitoes Cx.pipiens.

**Conflict of Interest:** We declare that there is no conflict of interest.

**Source of Funding:** Self

**Ethical Clearance:** Nil

**References**


Molecular Characterizations of a High Pathogenic Avian Influenza H5N8 in Iraq

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Abstract

High pathogenicity avian influenza subtype H5N8 viruses were detected in different areas in Iraq at the last 2017 and early 2018 and 2019 and the disease was detected clinically. This disease is responsible for high economic losses for poultry industry and threat human health, so that, this study was conducted for molecular detection, characterization and phylogenetic analysis of avian influenza in Iraq. AI subtype H5N8 is an infectious disease primarily in birds and responsible for severe respiratory illness which associated with a high percentage of morbidity and mortality in wild and domestic birds. During this study one hundred fifty different samples including (trachea, larynx and lung) were collected from different areas of broiler chicken from Baghdad and its surrounding regions during winter 2019. Avian influenza virus subtype H5N8 was detected by using real time RT-PCR technique, and specific kits (KyriR Germany) for AIV subtypes H5 and N8 were used respectively. The results revealed that (15) samples out of (150) collected samples were gave positive results for avian influenza H5, these positive samples were prepared for a second step of detection by RT-PCR test specific for N8 subtype the results revealed that only (12) out of (15) tested samples were positive for H5N8 (8%). Genetic sequencing of isolates and phylogenetic analysis of three selected isolates of (H5N8) bellowing to different areas indicate that all strains bellowing to H5 class (2.3.4.4) high pathogenic avian influenza revealed that they are closely related to Egyptian strain (A/duck/Egypt/ F446/2017. H5N8-MH893737.1) (with 97.6% identity). Analysis of the mono-basic amino acid (PQIEPR / GLF) at the hemagglutinin cleavage site revealed there is no deletion of the stalk region with the neuraminidase indicated that the isolates is a typical HPAI strain (A / duck / Egypt / F446/2017). The similarity of the nucleotide sequence analysis of hemagglutinin gene revealed that there was a high homology (97.6%) to that of A/duck/Egypt/F446/2017 H5N8.

Keywords: Avian influenza, Bird flu, Fowl plague, H5N8 avian flu

Introduction

Viruses of avian influenza are single-stranded RNA viruses, negative sense, segmented viruses classified by the Orthomyxoviridae family into groups A, B, C and D based on variations in their matrix proteins, internal nucleoproteins and antigenic characteristics, Influenza type A are only viruses have been known to have the ability to cause natural infections in birds based on their frequency and potential to cause illness in poultry1.

Influenza A / H5N1 was initially isolated from a Chinese goose in 1996. Humans infections were first recorded in Hong Kong in 1997 3. Informally, the avian flu was known as fowl plague or avian influenza and these viruses triggered a variety of influenza that adapted in birds 4. Avian flu is related to dog flu, swine flu, horse flu, and human flu, a disease caused by influenza virus strains that can be adapted in a specific species. Of the four influenza virus types (A, B, C and D), the influenza virus type A is a zoonotic disease and has an almost complete natural reservoir in avian5. Influenza viruses are typically categorized as surface proteins into a broad range of subtypes based

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aidabaral@yahoo.com
on 18 hemagglutinin HA (from H₁ to H₁₈) and 11 neuraminidase NA (from N₁ to N₁₁), some of which pose a threat to public health⁶.

Numerous specific subtypes 16 hemagglutinin subtypes from H₁ to H₁₆ and Nine neuraminidase subtypes from N₁ to N₉ bellowing to the avian flu viruses, but the H₁₇N₁₀ & H₁₈N₁₁ subtypes which are found in bats only⁷. Broad distribution of avian influenza viruses in different hosts may result in the exchange of their gene or gene fragments, which in turn contributes to highly antigenic variation and potential development of new strains of avian influenza viruses. This can cause significant epidemics and outbreaks, lead to tremendous economic losses in the poultry industry and may also pose a serious threat to human health⁶. The hemagglutinin gene plays a crucial role in the of influenza virus A life cycle, its involvement in recognition of receptor, attachment of virus particles on host cell, e fusion of membrane and entry inside the host cell⁷.

In 2014 the influenza/goose /Guangdong (1/1996) lineage clade (2.3.4.4) H₅N₈ avian influenza viruses with a high pathogenicity were originated in poultry and wild birds throughout Europe, Asia, and North America. At this time, the wild birds in the Netherlands were extensively investigated for HPAI H₅N₈ virus (real-time PCR targeting the M and H₅ genes) and antibody detection (inhibition of hemagglutination and neutral virus)¹¹. The rapid detection by RT-PCR of AIV subtypes H₅ has a crucial role for control of avian influenza disease infection, Pathogenicity of avian influenza infection was varies greatly depending several factor as a host species, virulence of strain, infective doses, and infection routs¹².

In Iraq, according to OIE (H₅N₈) struck again on a commercial farm near the Baghdad metropolitan area and it were killed 13,240 susceptible birds out of 29,000. The survivors were pulled out to control the virus spread, this detection makes Iraq’s sixth (H₅N₈) outbreak during 2018. avian influenza viruses with a high pathogenicity was identified during the last few years in several governorates and the disease was established at 2018 by the organization for animal health in the infected poultry with high sickness and high mortality rate, this reason lead to high economic losses for poultry industry with possible risk for human health¹³. Continuous surveillance of HPAI H₅ in Iraq is necessary to avoid economic losses in poultry industry and detection of outbreak points, so this study is the first to identified high pathogenic avian influenza H₅N₈. And it is aimed for genetic characterization and sequencing and phylogenic analyses of A1 subtype H₅N₈ and study the genetic variation between isolates, for these reasons this study was done.

**Materials and Methods**

Samples collection and screening:-

Between December 2018 and October 2019, 150 samples including (trachea, larynx and lung) were collected from different areas from Baghdad 75 samples, Dialla 42 samples, and Hilla 33 samples) from suspected poultry farm which given symptoms of avian influenza illness. These farms show a different symptom in poultry as ocular and nasal discharge, sneezing, enlarged infra-orbital sinuses. The mortality rate varies from 10% to 65% as well as subcutaneous hemorrhages, petechial hemorrhages on visceral organs and muscles, extreme inflammation, edema and red discoloration of the shanks and feet. Greenish diarrhea was common in badly affected birds, paralysis, and drooping of wings. These samples were collected in sterile container and send to specific diagnostic laboratory for confirmative RT-PCR test.

Samples preparation and RNA extraction:

A part of collected samples were pooled in a sufficient volume of sterile buffer (1 ml of normal saline), another parts were preserved for molecular and histopathological tests. Samples were soak for an adequate period of time and finally wash out the sample by plus vortexing, RNA from samples were extracted from tissues by using kit of the total RNA extraction (Kyli RNA extraction Kit) after extraction the RNA was elute in 60 µL of RNase-free D.D water, then store after adding 20 U of RN as inhibitor at (-80 ºC) until using for Real-Time TR-PCR.

Subtyping of isolates by using Real-Time PCR:

To differentiate influenza isolates from other non-influenza. real-time RT PCR specific for influenza A virus that amplifies the cleavage site of hemagglutinin gene coding sequence which performed using total of 800 bp RNA according the WHO guidelines⁸. Then the
positive isolates for influenza A test were further tested to detect the H₅ by using Real-Time PCR, then using a positive samples for detect the type of N₈ gene according to instruction of manufacturer company (AniCon Labor GmbH) Kit (Germany).

**Sequencing and phylogenetic analysis (incl. comparison to NCBI database):**

A 800 bp fragment containing the cleavage site of the HA gene of Influenza A type H₅N₈ has been sequenced and was phylogenetically analyzed based on comparison to known reference strains by (AniCon Labor. GmbH Emstek, Germany). Influenza viruses were known via a database that was created by downloading influenza virus sequences from the (NCBI) information. Then, tested in the influenza virus database by Basic Local Alignment Analysis Tool for nucleotides (BLASTn).

**Results**

Molecular detection of avian influenza H₅N₈ and Real-Time PCR:-

From about 150 collected samples 15 positive samples that mean an avian influenza virus was present in many area of country in this collection period. In real-Time PCR 15 positive samples of avian influenza A virus and only 12 samples were give a positive result for H₅N₈ (about 8%) by using H₅ kit and N₈ respectively from (KyltR) company for veterinary diagnostic kits.

Based on the amplified sequence of nucleotide coding for the hemagglutinin glycoprotein of Influenza A Virus (type H₅), the RNA extracted from sample numbers A1910987.001, A1910987.002 and A1910987.003 belongs to HPAI-H₅ Clade (2.3.4.4) and is most related to strain A/duck/Egypt/F446/2017(H5N8) (MH893737.1) (each 97.6%).

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Sample ID</th>
<th>Assay Result</th>
<th>Sample Type</th>
<th>Ct</th>
<th>Endpt</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1T</td>
<td>Positive</td>
<td>SPEC</td>
<td>21.5</td>
<td>756</td>
</tr>
<tr>
<td>A2</td>
<td>2T</td>
<td>Positive</td>
<td>SPEC</td>
<td>29.6</td>
<td>486</td>
</tr>
<tr>
<td>A3</td>
<td>3T</td>
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<td>SPEC</td>
<td>29.4</td>
<td>420</td>
</tr>
<tr>
<td>A4</td>
<td>4T</td>
<td>Positive</td>
<td>SPEC</td>
<td>32.4</td>
<td>320</td>
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<td>A5</td>
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<td>SPEC</td>
<td>26.6</td>
<td>661</td>
</tr>
<tr>
<td>A6</td>
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<td>1</td>
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<td>7T</td>
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<td>SPEC</td>
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<tr>
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<td>SPEC</td>
<td>38.1</td>
<td>69</td>
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<td>SPEC</td>
<td>37.4</td>
<td>116</td>
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<td>1</td>
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<tr>
<td>A11</td>
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<td>2</td>
</tr>
<tr>
<td>A12</td>
<td>12 Lung</td>
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<td>SPEC</td>
<td>36.2</td>
<td>143</td>
</tr>
<tr>
<td>A13</td>
<td>13 Larynx</td>
<td>Positive</td>
<td>SPEC</td>
<td>38.1</td>
<td>85</td>
</tr>
<tr>
<td>A14</td>
<td>14 Larynx</td>
<td>Positive</td>
<td>SPEC</td>
<td>21.7</td>
<td>88</td>
</tr>
<tr>
<td>A15</td>
<td>15 Lung</td>
<td>Positive</td>
<td>SPEC</td>
<td>18.4</td>
<td>82</td>
</tr>
</tbody>
</table>
Figure 1:— Real Time PCR amplification of RNA from samples infected with avian influenza H5.

Table 2: A positive samples that give high titer of H5 (and low CT value) were selected and used for detection of N8 by using specific kit for neuraminidase:

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Sample ID</th>
<th>Assay result</th>
<th>Sample type</th>
<th>Ct</th>
<th>EndPt</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1T</td>
<td>Positive</td>
<td>SPEC</td>
<td>18.3</td>
<td>482</td>
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<tr>
<td>A10</td>
<td>2T</td>
<td>Positive</td>
<td>SPEC</td>
<td>26.5</td>
<td>294</td>
</tr>
<tr>
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<td>3T</td>
<td>Positive</td>
<td>SPEC</td>
<td>29.2</td>
<td>145</td>
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<tr>
<td>A12</td>
<td>4T</td>
<td>Positive</td>
<td>SPEC</td>
<td>32.2</td>
<td>76</td>
</tr>
<tr>
<td>A13</td>
<td>5T</td>
<td>Positive</td>
<td>SPEC</td>
<td>28.2</td>
<td>181</td>
</tr>
</tbody>
</table>

Table 3: Sequencing and phylogenic analysis (incl. comparison to NCBI database):
Method: H- & N-specific Real-Time RT-PCR (Kylt® Influenza A - H5 / N1) (a)

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Sample description</th>
<th>CT.H5</th>
<th>CT.N1</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
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<td>FTA-card(spot 1)</td>
<td>24.3</td>
<td>-</td>
<td>H5 positive</td>
</tr>
<tr>
<td>A1910987.002</td>
<td>FTA-card(spot 2)</td>
<td>31.8</td>
<td>-</td>
<td>H5 positive</td>
</tr>
<tr>
<td>A1910987.003</td>
<td>FTA-card(spot 3)</td>
<td>31.8</td>
<td>-</td>
<td>H5 positive</td>
</tr>
</tbody>
</table>

Figure 2: explain the phylogenetic analysis and sequences hemagglutinin of influenza A virus subtype H5N8 of the detected tissues samples from birds in Iraq. The phylogenetic tree was built using the neighbor-joining method. Within the research was included the representative of viral sequences which were quite close to those recorded in this review. These labels the H5N8 viruses identified in this study. viral sequences are provided. Scale bar indicates estimated genetic distance belongs to HPAI H5 Clade 2.3.4.4 and is most related to strain A/duck/Egypt/F446/2017(H5N8) (MH893737.1) (each 97.6\%).
Discussion

Because of lack of the continuously surveillance of avian influenza subtype H5 in some countries, therefore H5 avian influenza viruses are still circulating in different areas of the world. So that, continuous surveillance of the domestic and wild birds and annually genomic analysis of high and low pathogenic avian influenza viruses by different methods such as next-generation sequencing is recommended due to the high mutation rate of influenza viruses which has been determined by the sequencing. Real time RT-PCR technique was used to detect avian influenza from collected samples by amplification of hemagglutinin gene. The results of sequencing of three selected positive local isolates (A1910987.001, A1910987.002, A1910987.003) for hemagglutinin gene by obtaining a 800 bp fragment containing the cleavage site of the HA gene of Influenza A type H5 was disagree with which was found that H5H8 isolated in this year was bellowing to clade 2.3.4.4 group B and clustered with isolates from Iran and Belgium. The genetic sequences of isolates indicated that there were a similarity between the local isolates and isolate from Egypt and Saudi Arabia. Genetic analysis of AIV H5N8 virus was indicates that the virus probably reach to Iraq by wild birds migration due to the position of Iraq as a road of migratory pathway of birds, other several outbreaks of AIV (H5N8) virus infections was reported in Egypt, Iran and Saudi Arabia at the end of 2017 and 2018 and it cause exponentially increasing of mortality in flocks of birds with high economic losses. The location of isolates at the end of phylogenetic tree is fits with the hypothesis route of (H5N8) virus was introduce into Iraq via the migratory bird but not through the direct contact with infected birds, because the viruses when detected there is no active direct trade and contact with other infected countries and before this time the virus was never been detected in the country. In conclusion the Iraqi HPAI H5N8 isolated detected in this study were belonged to (A/duck/Egypt/F446/2017(H5N8)) with (97.6%) identity and it may contain virulence-enhancing properties to infect the mammalian host as a result of its continuous reassortment of it is genes, for this reasons the identification and monitoring of both low and high pathogenic viral strains is important for the early management and control of avian influenza viruses. Although the AI H5N8 virus was not detected so far in humans, we should remain aware of the potential of this virus to be transmitted from the avian host to the human population.

Ethics Approval and Consent to Participate:-

The study was conducted following the ethical guidelines of the Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad, and consent to collect samples from the infected animals was provided by the owner of the farm. After confirmation that influenza infection has spread in the farm.

Source of Funding – Self

Conflict of Interest – Nil

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Novel Molecular Detection of BPV-10 and BPV-11 Genotypes in Al- Anbar, and Baghdad Provinces

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Abstract

In this study, the prevalence of diverse bovine papilloma viruses in cutaneous lesions was investigated in Iraqi cows. Fourteen specimens were taken from udder growths of Iraqi dairy cows, which demonstrated rice grain-like and small, raised flesh-like appearance. DNA was extracted from the teat wart samples, then was amplified by the polymerase chain reaction (PCR) using primers against BPV-10 (403 bp) and BPV-11 (560 bp). PCR results revealed mixed infections of BPV-10 and BPV -11 in four samples, while the remaining ten samples were infected with BPV-10 only. These findings demonstrate the presence of bovine papillomaviruses 10 and 11 in rice grain-like cutaneous lesions on the teats of cows and multiple elevated growths on udder. The detection and report of the BPV-10 and BPV -11 genotypes in the current study is for first time in Iraq, which are known in the Middle East region, are crucial for disease control measurements.

Keywords: BPV-10, BPV-11, Genotypes, Molecular detection, PCR

Introduction

Cutaneous warts are spread between cattle and originate from the bovine papilloma (BPV) ¹, of which there are multiple strains. The lesion can generally be described as benign, yet may turn malignant ². BPVs exhibit tropism for mesenchymal and mucosal tissue as well as squamous epithelium ³-⁶. Studies have identified fourteen virus genotypes that have been grouped into four genera: Dyoxipapillomavirus, Deltapapillomavirus, Epsilonpapillomavirus, and Xipapillomavirus ⁷-¹⁰. BPV-10 and BPV-11 belong to the Xipapillomavirus family, which contains another type of BPV like viruses ⁵, which are associated with the production of true papillomas ¹¹.

BPV is naked, icosahedral, and possesses double-stranded circular DNA that is 8000 base pairs (bp) containing 5 or 6 open reading frames (ORFs) that are expressed early during infection. Besides two ORFs that are expressed late during infection, the genetic material is organized into three regions: early domain, a long control domain, and a late domain, within which diverse necessary and vital proteins are encoded ¹². With cofactors such as environmental carcinogens present, BPV-induced benign lesions degenerate naturally, but may also develop into cancer ⁴. Clinical, histopathological, and immunohistochemical examinations are all usually used in BPV diagnosis ¹³. Molecular characterization of bovine papillomavirus in Iraqi cattle showed the widespread presence of this oncogenic virus and the contribution it has to the development of skin tumors. BPV1, 2, and 13 affect dairy and beef cattle and induce remarkable commercial losses ¹⁴,¹⁵. This study aims to investigate and identify the etiology of teat and udder warts of Iraqi dairy cattle using molecular and histopathological approaches.
Approaches and protocols

Biopsies

Fourteen teat cutaneous papilloma samples were taken from bovine farms in the Al-Anbar and Baghdad province, including some brought to private clinics between August 2015 to May 2017. The samples that were collected demonstrated a variation in diameter range, about one to three centimeters, and collected from various portions of the teat and udder (Figure 1). For samples that would undergo molecular biology analysis, deep freezing was undertaken, whilst 10% neutral buffered formalin were used to fix samples undergoing histopathological analysis.

Histological study:

Teat and udder specimens were fixed and processed according to routine protocols, as described previously (16). After fixation in 10% NBF, tissue fragments were processed with xylene, dehydrated using ascending concentrations of ethanol (70 to 100%), and set in paraffin, after which they were incised to 5-m pieces, mounted into the slides, and stained by hematoxylin and eosin (H&E) and observed by veterinary pathologist under light microscopic and photographed.

DNA extraction:

Automated DNA extraction system was used to extract genomic DNA (Magnesia, Anatolia Geneworks, Turkey) was utilized in this analysis following the manufacturer’s instructions. The -80 degree freeze samples were processed via an automated Magnesia DNA Extraction machine.

BPV-10 and BPV-11 Primer designing

Primers were designed by aligning the viral genome by the ApE program (http://biologylabs.utah.edu/Jorgensen/wayne/ape): and NCBI Primer-BLAST. These specific primers for genotyping were made by selecting conserved regions for each genotype specifically designed for BPV-10 and BPV-11. Primer sequences are shown in Table 1. The primers were designed to target conserved regions for alignment of BPV-10 (BPV-10, AB331651, and BPV-10 isolate KF017607, MYP55) and BPV-11 complete genome (BPV-11 DNA, AB543507) by the use of the ApE program. The designed primers were tested for inclusivity and exclusivity.

### Table 1 Primers for BPV-10 and BPV-11

<table>
<thead>
<tr>
<th></th>
<th>BPV-10 (403bp amplicon)</th>
<th>BPV-11 (560 bp amplicon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>forward</td>
<td>5'-GGACAAATGGCACCAGGGGTA-3</td>
<td>5'- GCCTGCAACTAGGGTCTCTG -3</td>
</tr>
<tr>
<td>reverse</td>
<td>5'-CTGTGGTACGACGCTGGAGT -3</td>
<td>5'- TAGATCGCGATGACGACTGC -3</td>
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</tbody>
</table>

PCR Amplification

PCR was done utilized a Thermal Cycler (SureCycler 8800, Agilent Technologies, USA) with each individual reaction prepared to a final volume of 25-L containing the following:

<p>| | |</p>
<table>
<thead>
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<th></th>
<th></th>
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<tbody>
<tr>
<td>100–250ng DNA,</td>
<td></td>
</tr>
<tr>
<td>2mM MgCl2,</td>
<td></td>
</tr>
<tr>
<td>1.25-L primers,</td>
<td></td>
</tr>
<tr>
<td>(0.5-M), and 12.5-L 1X Abm HotStart Ready-mix (Applied Biological materials, Canada).</td>
<td></td>
</tr>
</tbody>
</table>
The PCR assay procedure included the following

<table>
<thead>
<tr>
<th>Process</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting denaturation</td>
<td>94°C</td>
<td>180s</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>20s</td>
</tr>
<tr>
<td>Annealing</td>
<td>44°C</td>
<td>15s</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>30s</td>
</tr>
<tr>
<td>35 Cycle Extension, final step</td>
<td>72°C</td>
<td>10 minutes /kb</td>
</tr>
</tbody>
</table>

BPV DNA PCR products were identified by a 1.5% agarose gel containing ethidium bromide electrophoresis assay. Gels were put in TBE buffer and ran at a fixed voltage of 100V for thirty-five minutes and visualized via a gel Documentation System (VISION, Scie-Plas, UK). Clinically healthy slaughtered cattle DNA extracted from skin tissues were used as negative control.

**Results**

Clinically diagnosed teat wart cases in the dairy cows, demonstrated a macroscopic appearance as rice grain-like, small, flesh-like growths.

These were generally diffused and enriched with vascularity (see Figure 1A and B). Gross examination revealed that skin warts were exophytic. The histopathological study describes the tumors as cutaneous papillomas (benign squamous cells). Microscopically, all tumor growths showed comparable histological characteristics; according to different degrees of parakeratosis or hyperkeratosis with the presence of papillary-like projections of the squamous epithelium (Figure 2A and B).

In all tumor specimens assessed, most keratinocytes had a clear halo surrounding the nuclei, while others exhibited a pyknotic nucleus that is described as koilocytes (Figure 2C). Presence of koilocytes that have severely condensed pyknotic nucleus with basophilic staining, in addition to multiple degenerated keratinocytes (Figure 2D).

![Figure 1](image1.png) A) Cow suffering from teat papilloma. B) Cow suffering from multiple udder Papilloma
Figure-2. Histopathological characterization of teat and udder warts in a cow. A) Presence of papillary-like projections of the squamous epithelium. Stratum corneum showed a hyperkeratosis (black arrow), H&E, 40x. B) The proliferation of the squamous epithelium that has koilocytes can be observed H&E 100x. C) Hyperkeratosis and presence of clear halo surrounding the nuclei of most keratinocytes H&E 400x. D) Presence of koilocytes that have severely condensed pyknotic nucleus with basophilic-staining. In addition to multiple degenerated keratinocytes (black arrow). (H&E, 400x).

Bovine papillomavirus type 10 (BPV-10) DNA was discovered exclusively in ten (71.42%) of the fourteen teat and udder wart samples (Figure 3, Table 1), whilst four samples (28.58%) showed mixed infections with BPV-10 and BPV-11 (Figure 4, Table 1) which belong to Xipapillomavirus family (8). These BPV-10 and BPV-11-positive samples yielded two DNA fragments of 403bp and 560bp, respectively, in length for E2, E5, and L2 genes. The PCR produced another DNA fragment of 556 bp, of BPV_11 gene in 4 udder teats samples that were amplified utilizing specific gene primers.

Figure-3. PCR products for BPV-10 genotypes pictured in an ethidium bromide-stained 1.5% agarose gel following electrophoreses in TBE buffer. M: 100–10,000 bp marker; lanes: 1–8 BPV-10 positive samples (Teat wart samples) with band sat 403 bp; lane NC: negative control.
Table-2, showing PCR results of animals infected with teat and bovine udder papillomatosis.

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Age of animal (years)</th>
<th>Location of the warts</th>
<th>PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Small growth on udder and teat</td>
<td>BPV-10&amp;BPV-11</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
<td>Small fleshy growth on udder and teat</td>
<td>BPV-10&amp;BPV-11</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>Small fleshy growth on udder and teat</td>
<td>BPV-10&amp;BPV-11</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Small fleshy growth on udder and teat</td>
<td>BPV-10&amp;BPV-11</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>Teats</td>
<td>BPV-10</td>
</tr>
</tbody>
</table>

Figure-4, PCR products for BPV-11 genotypes visualized in an ethidium bromide-stained 1.5% agarose gel following electrophoreses in TBE buffer. M: 100–10,000 bp marker; lanes: 1–4 BPV-11 positive samples (udder and teat wart samples) with bands at 560 bp.
It is clear from the above that PCR experiments ultimately showed mixed infections of viral DNA of BPV-10 and BPV -11 in four samples, and ten teat samples showed BPV-10 only (Table 2).

**Discussion**

Figures 1 and 2 show the distinct appearance of benign skin tumor and multiple stages of hyperkeratinization in addition to other tumor related microscopic and gross changes in cutaneous layers. Previous studies by our team for different types of BPV (type 1, 2 and 13) showed that tumor samples were cutaneous fibropapillomas, which demonstrated the main features of papillomatosis (14,15). PCR products for BPV-10 genotypes Fig.3 were detected with band sat 403 bp in agreement with 17 while PCR products demonstrated BPV-11 genotypes with band sat 560 bp (Fig. 4) in agreement with (18). To summarize, PCR results identified either BPV-10 alone or BPV-10&BPV-11 (Table 2), with ten samples showing exclusivity to BPV-10 whilst four demonstrated a mixed infection of both BPV-10 and BPV-11. In brief, clinically diagnosed cases of the teat and bovine udder papillomatosis were studied to recognize BPV-10 and BPV-11 genotypes, which were prevalent mostly in the Middle part of Iraq that is the primary site for large cattle-raising farms. The detection and identification of BPV-10 and BPV-11 genotypes in this geographic area are crucial in effective disease control. In conclusion, Bovine papillomatosis of teat and udder were confirmed in this study to be initiated by BPV-10 and BPV-11 genotypes in the Middle regions of Iraq, where cattle farms are spread, which will ease the control requirements of the disease in the area.

**Ethical Approval**

Consent was obtained from the owner of farm animals from which samples were gathered. Approval was obtained from the scientific committee at The Biotechnology and environment research center, University of Fallujah, Al-Anbar, Iraq. The experimental in vitro part was accomplished at the department of Experimental Therapy, Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University, Baghdad, Iraq

**Acknowledgment:** The authors thank Dr Emyr Bakker and Dr Israa N. A. Al-Ibadi for their assistant in the English editing and improving the manuscript language. We would like to thank the support of Mustansiriyah University, Iraqi Center for Cancer and Medical Genetic Research, Baghdad, Iraq.

**Conflict of Interest:** nil

**Source of Funding:** nil

**References**


Stimulation of *Staphylococcus aureus* Ligase Enzyme by Magnesium Ion

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**Abstract**

Ligase enzymes were discovered as a member of the nucleotidyl transferase family. Here in this paper, DNA Ligase is extracted from *S. aureus* works with the cofactor NAD⁺ to make a phosphodiester bond and reform between the 3’hydroxyl and 5’phosphate DNA end. *Staphylococcus aureus*-DNA Ligases Enzyme type A (SLE-A) contains two essential domains; NTase and OB- fold domain, which are the most essential domains for the enzyme function. The main aim of the study is to investigate the activity of SLE-A in the presence of magnesium ion (Mg²⁺) by evaluating several kinetic parameters on a time course. The result showed that SLE-A has optimal activity at 500 µM of Mg²⁺. Furthermore, the low number of Equilibrium Association Constant (Kₘ value) explains the binding affinity between DNA ligase of *Staphylococcus aureus* SLE-A enzyme and Mg²⁺ ion was very high and sold.

**Keywords:** DNA ligases, Magnesium ion, Staphylococcus aureus, Michaelis-Menten equation.

**Introduction**

**Background of DNA ligase.**

Fifty years ago, Lehman and other groups discovered the DNA ligases and was a turning point event in molecular biology (1-4). Other DNA ligases had been lighted on since and they are presented in all domains of life (5). The DNA ligase enzyme is an important component in the Ligation reaction, which also called DNA seals, DNA joins and/or DNA fixing enzyme (6). All types of DNA Ligases which have the same protein fold are considered as part of nucleotidyltransferase family. For example, DNA ligases and RNA ligases (7), they participated in multi processes for instance DNA repair, recombination and replication (8). DNA ligase enzyme plays an important role during DNA replication: Firstly, joins Okazaki fragments, in addition it carry out a several DNA repairs like base excision, nucleotide excision, single and double-stranded repairs (9). The lake or mutation of SLE-A (referees to LigA gene), resulted the loss of the ability to ligate Okazaki fragments during the replication steps (10). DNA Ligases are very potent chemical material in the development of biotechnology, bimolecular and genetic engineering (11). Another important implication, is that NAD⁺-dependent ligases not present in mammalian cells as well as they are involved in developing antibacterial drugs “A requirement for an antibacterial enzyme target is that it should be essential for the organism and not present in the host (12). The formation of a phosphodiester bond between adjacent 5 phosphate and 3’ hydroxyl DNA termini is catalyzed by Ligases which known to fix several substrates.

**Indeed, ligases are participated in the base**

Excision DNA repair, culminating step of nucleotide and the ligation of Okazaki fragments during cell replication (13-14). Importantly, NAD⁺ ligases play an important role in cell survival for prokaryotes (15). The main characteristics of DNA ligase in prokaryotic is to present into all bacteria and all bacterial species contain
LigA protein\(^{(16)}\). Kaczmarek announced in 2001, that the deletion or mutation of the LigA gene in *Staphylococcus aureus* (represent to SLE-A) lead to loss the complete growth in the bacteria \(^{(17)}\). The *S. aureus*-DNA ligase enzyme A (LigA) is formed of 666 signal amino acid and consist of 6 main domains with 74,993 Da molecular weight. The tertiary structure of *Staphylococcus aureus* of LigA (SLE-A) protein consists of two main domains called nuclotidyltransferase/NTase domain and an Oligomer Binding (OB domain), which are considered as a catalytic core for all domains of life \(^{(18-19)}\).

**Magnesium Ion in DNA Ligase.**

One of the most important cofactor in the ligation or the mechanism process of DNA ligases enzymes is MgCl\(_2\) Ion. It considers a second cofactor in the reaction of ligase. Mg\(^{2+}\) is a co-factors that allows the ligase enzymes to join and shut a nick at the backbone of Deoxyribonucleic acid. Furthermore, LigA DNA ligase needs Mg\(^{2+}\) to draw the AMP groups that is very necessary during the mechanism of ligase and attaches with the active site of amino acid called lysine to do its work. The AMP groups comes when the ligase enzyme touches with cofactor ATP or NAD+ to form a phosphoamide-linked AMP and without the NAD\(^+\) or ATP the reaction of ligation will be stopped completely \(^{(9)}\).

**Materials and Methods**

**The Cloning of *Staphylococcus aureus* DNA Ligase.**

The published open-reading frames of *S. aureus*-DNA Ligase enzyme A (SLE-A) (belongs to LigA) was retrieved from the NCBI-PubMed database using Gene ID 45575143 and the gene size is 2004 base pair for *S. aureus* LigA. Gene was synthesised (GeneArt, ThermoFisher, UK) with a 20 bp flanking sequence containing an Ndel site and cloned into the Ndel site of pET29c. Kanamycin-resistant transformants of *S. aureus* LigA were screened by colony PCR and those showing the requisite sizes were sequenced in full on both strands (Genewiz, UK). Figure 1 is shown a sample of *S. aureus* (SLE-A) gene was run on a 1% agarose gel. Large-scale preparations of each plasmid were made (Qiagen, UK) and stored at \(-20^\circ\)C in 50 µl aliquots. DNA primer sequences are as follow as (green colour refers to the NdeI site of pET29c and black colour refers to the forward and reverse primers of *S. aureus*-DNA ligase enzyme type A gene.):

\[
\text{LigA}_{\text{top}}: \\
\text{CATATGGCTGATTATCGTCTCG} \\
\text{LigB}_{\text{bot}}: \\
\text{CATATGCTAACTATTTAATTTCATTTT}
\]

![Figure 1: Cloning of *S. aureus*-DNA ligase enzyme type A.](image-url)
1% of agarose gel showing the PCR cloning from *S. aureus*-DNA enzyme type A as follows: Lane M – 1 kbp DNA ladder (NEB), showing sizes of DNA between 500 bp to 10,000 bp. Lane 1 is a colony PCR products of SLE-A gene, the length of *S. aureus* SLE-A (LigA) is 2004 bp depending on the GeneBank plus the sequencing of plasmid.

The expression and purification of *Staphylococcus aureus* DNA Ligase.

For *S. aureus* LigA (SLE-A) expression, competent *E. coli* BL21 (DE3) cells was transformed with 0.5 µg plasmid (pLigA) and grown overnight at 37°C on LB-agar containing 50 µg/ml kanamycin. A single colony was picked and grown overnight in an orbital shaker at 37°C in 5 mL LB media. A 1 mL aliquot of this starter culture was added to 500 mL fresh LB broth and grown under the same conditions until an OD$_{600}$ of 0.6 was reached. Protein expression was induced with 1 mM isopropyl B-D-thiogalactoside (IPTG) and the culture incubated at 37°C for 2 hours (LigA) until an OD$_{600}$ ~2.0 was reached. Bacterial cultures were harvested by centrifugation at 6000 rpm for 20 minutes at 4°C and the media discarded. The cell pellet was re-suspended in 15 ml Lysis buffer (10 mM sodium phosphate, pH 7.0, containing benzamidine and PMSF protease inhibitor cocktail (Sigma-Aldrich, UK) was sonicated on ice in 10 second pulses over 3 minutes and spun at 18000 rpm for 20 minutes at 4°C to separate supernatant and cell debris. Ligase A was subsequently purified from the supernatant using a two-step method; all steps were at 4°C. In the first step, NaCl was adjusted to 1M in order to disrupt protein-nucleic acid interactions and ammonium sulphate then added slowly over two hours to a final concentration of 35% (w/v) in order to precipitate the enzyme. Following centrifugation at 18000 rpm for 20 minutes, the pellet was resuspended in an appropriate volume of Buffer A (10 mM sodium phosphate, pH 7.0). Salt was removed by membrane dialysis overnight in Buffer A. In the second stage, enzymes were injected onto a Hi-Trap heparin anion-exchange column (GE Healthcare, UK) in Buffer A and eluted in the same buffer with NaCl gradient to 1 M. Fractions eluting from the column were analysed by SDS-PAGE to confirm the presence and purity of the protein. Fractions containing purified LigA was pooled and dialysed into Buffer A. Ligase activity assays were then used to check for correctly-folded, functional ligase. LigA was further purified using size-exclusion chromatography (GE Healthcare, UK).

Fractions containing purified LigA were pooled and concentrated using spin column to ~ 1 ml. *S. aureus* LigA was finally dialysed into 1×Ligase storage buffer (30 mM Tris-HCl, pH 7.2, 1 mM DTT and 50 µg/ml BSA and 30% (v/v) glycerol). Aliquots of 50 µl were stored at -20°C and used fresh for subsequent kinetic and binding assays.
**Figure 2** is shown the large scale of expression of DNA ligase (LigA) and the final purification of the *S. aureus* SLE-A protein.

![Image of SDS PAGE gel](image)

10% SDS PAGE gel shows the large induction of *S. aureus* SLE-A enzyme in *E. coli* BL21 (DE3) as follow: Lane M shows an NEB protein marker indicated by sizes (in kDa) pointed to the left of the gel. Lanes 1 and 2 shows the total post-induction of expressed SLE-A. Lane 3 shows the corresponding samples of *S. aureus* LigA protein that pooled from heparin column with molecular weight (size) 74,993 Da. Lane 4 shows the final purification of pure protein via using size exclusion column.

**Ligase activity and timecourse assays.**

Three HPLC-purified oligonucleotides (Fisher Scientific, UK) were used:

- **20Top** 5’-HEX-ATCTCGCGTATGGGCCTTCG-3’
- **30Top** 5’-P-CTGCTCACAGGACACCTGGTATACGTAATG-3’
- **50Bot** 5’-CATTACGTATACCAGGTGTCCTGTGAGCAGCGAAGGCCCATACGCGAGAT-3’
These were mixed in a ratio of 1:1:1 at a concentration of 10 µM in 50 µl of 1×Ligase reaction buffer (30 mM Tris-HCl, pH 7.5, 1 mM DTT and 50 µg/ml BSA) and annealed by cooling in a PCR machine from 100°C to 4°C at 0.1°C/min. The resulting 50mer oligoduplex contained a single off-centre nick between the 20Top and 30Top strands with a phosphoryl group on the 5’-side of the nick; a hexachlorofluorescein (HEX) group at the 5-end of the 20Top strand permitted the DNA to be quantitated. Kinetic assays on LigA was carried out in 1× ligase reaction buffer. The concentration of nicked oligoduplex was 1000 nM in most experiments. Each timecourse (50 µl) contained nicked oligoduplex, 1×Ligase buffer, the requisite cofactor(s) and 40 nM of LigA. Each reaction of time course was containing a single nick of six MgCl₂ samples ranging from (0.1, 1, 10, 50, 100, and 500 μM) on a denaturing PAGE 15%. All the samples were incubated at 16 °C. The time points were prepared for 5 times as follow (0 time, 3 mins, 5 mins, 10 mins, 20 mins and 60 mins) and all the tubes were heated at 100 °C for 2 mins. Shortly, the intensity of the 50TOP bands were measured by using computer software ImageGauge. The data were plotted with % intensity against time revealing the initial rate.

**Data Analysis.**

Products of timecourse reactions were run on 40 cm long, 0.4 mm thick 15% (w/v) denaturing polyacrylamide gels containing 1×TBE (89 mM Tris-borate, 2 mM EDTA) and 40% urea. Gels were pre-run at 60 W for 30 minutes until warm. Samples (10 µl) were heated at 90°C for 2 minutes, loaded and run at 50 W for ~90 minutes. The HEX-labelled DNA strands were visualised by excitation at 600 nm using a fluorescence imager (FLA-5000, Fuji, Japan). The digitised images were quantified using ImageJ (imagej.nih.gov) by firstly removing background (50-pixel ‘rolling-ball’ average) and then integrating the area under each peak. These data were used to calculate the fraction of counts distributed between the 20mer substrate and 50mer product bands in each lane; these were multiplied by the DNA concentration to give the amount of each in nM. Initial rates (V₀) were determined by the gradient of the first 20% of the reaction. Rate data were fit to the Michaelis-Menten equation (Grafit v5, Erithacus Software, UK) and Vₘₐₓ and Kᵣ parameters were elucidated by plotting their V₀ values for each timecourse and fitting them by using Michaelis- Menten equation as follow: \[ V₀ = V_{max} + \frac{[S]}{K_m + [S]} \]

The definition of Kₐ is the concentration of substrate that gives half maximum rate. It was obtained from the Michaelis- Menten equation fit above as well.

**Results**

**Nick-joining ligation activity.**

All proteins purified in this study have been proved to join nicks in double stranded nucleic acid in different conditions and terms. The purified of LigA protein of *E. coli* DNA ligase have been confirmed to join nicks in double stranded nucleic acids with varying efficiencies in presence of NAD⁺. In order to achieve the ligation experiment and how the ligation was affected by varying concentrations of Mg²⁺, a double-stranded DNA substrate (dsDNA) was synthetised from Fisher Scientific, UK with a single nick on the top strand. Three HPLC purification of oligonucleotides were synthetised as follow: the length of first top oligonucleotide was 50 bases contains from two top strands (20 and 30 bases) and both of them are complementary to adjacent the 50 base bottom strand. The 20 top single strand attached fluorescent HEX group and the nick was made on the top strand between 30 top and 20 top. The complementarity in the bottom and top strands location, the two top strands (30 and 20) was adjacent to each other. The nick on the top strand (50 oligonucleotide) was at 3’-hydroxyl group of the 20 base strand and 5’-phosphate group of the 30 base strand.

When the buffer has the nicked DNA substrate and SLE-A (LigA) protein of the appropriate concentration and conditions, the nick on the top strand will be sealed by the proteins.

The samples were taken at specific time points and transferred to the stop buffer to stop the ligase protein its work. During electrophoresis, the denaturing gel separate the double stranded structure (50 base top and bottom) and causes to unfold the dsDNA into the linear chain. The dsDNA substrates will be migrated through the gel as single stranded. Therefore, the size of DNA ligation is easy to distinguish as the distance migrated by the DNA. Once the products sDNA of 20 and 30 base strands ligated will be migrated less position on the
denaturing gel, comparing to only the 20 base strand, which will be stayed at the bottom of the gel. The visible light to the ligated products in the denaturing gel was easy to follow due to the attached of fluorescent HEX group at 20 top strand.

The Effect of Mg$^{2+}$ ion on the S. aureus DNA ligase.

To assess whether ligation of such nucleic acids may be a substantial value of these enzymes, at least in biochemical condition, all these experiments have been characterized the ligation of the dsDNA with different protein of S. aureus SLE-A (LigA) across a range of independent variable of Mg$^{2+}$ ion. All the experiments were carried out at 16 °C (except temperature) and 1 µM of oligonucleotide substrate in total volume of 50 µl of 1X DNA ligase buffer and the mixture of reaction were completed with free water. The concentration of enzymes that used in all these experiments was 40 nM. To check how was influenced by different Mg$^{2+}$ ion concentrations; the process of ligation was performed in the existence of Mg$^{2+}$ concentrations from 0.1, 1, 10, 50, 100, and 500 µM. At low of Mg$^{2+}$ ion, initial average increases slowly till over 10 µM (does not show), where is initial rate rises rapidly around 50 µM and the loop of curve begins to rise until 500 µM. The value for $V_{\text{max}}$ gained by organized initial average in front of Mg$^{2+}$ ion concentrations. The $V_{\text{max}}$ obtained by Mg$^{2+}$ ion concentrations which was µM/min and the $K_m$ (the $[\text{MgCl}_2]$ that given half $V_{\text{max}}$ rate) was as well in µM. To examine the $V_{\text{max}}$ and $K_m$ (shown as $K_d$ at the figure) for Mg$^{2+}$, using six Mg$^{2+}$ concentrations, these initial rate values (in % per minute) were calculated against the concentration of Mg$^{2+}$ in each experiment and fit the curve to a Michaelis-Menten equation by using the software Grafit. The initial rate and the measurement of the intensity were explained by details in the data analysis in the Material and Method above. Figures 3 is an example of gels showing the ligation of DNA ligase of S. aureus SLE-A with different concentration of Mg$^{2+}$ that appeared by bands of DNA in the denaturing gel. The plot for the MgCl$_2$ ion shows below in Figure 4. The $V_{\text{max}}$ (capacity) of Mg$^{2+}$ ion was 3.9 %/min where is the $K_m$ (is shown as $K_d$ ) was 5.2 µM.

![Denaturing PAGE gel](image)

**Figure 3** is the denaturing PAGE gel of three of the six MgCl$_2$ Ion concentrations tested.

Denaturing PAGE gel that was got from this study is labelled as following lanes: 1-6 which is corresponded to the following time points 3,5,10,20 and 60 minutes, at 500 µM of Mg+$^{2+}$ ion as an example of this gel, at the top of gel are 50 mer (50 Top) and the bottom of gel are 20 mer (20 Top). The Bromophenol blue is the colour which is used to check the bands on the denaturing gel.
Discussion

Because of the NAD\(^+\)-dependent DNA ligase is very essential enzyme for DNA replication and repair, it had been reported as a prospective broad-spectrum antibacterial target, since there are a high conserved phylogenetic and distinctly different from the Eukaryotic DNA ligases \(^{20-22}\). Thus, prokaryotic NAD\(^+\)-dependent ligases is present as a promising drug target for antimicrobial therapy comparing to ATP-dependent in human \(^{23-24}\). In 1973s, Modrich and Lehman were referred that magnesium ion increase the activity of ligase protein. Magnesium ion is very important component in the step1 and step2 of the ligation mechanism of ligases enzyme in general. Moreover, in the same study showed that the magnesium ion is being involved to increase the rate of phosphodiester synthesis and DNA adenylation in the system of mechanism of ligases enzymes \(^{25}\). The obtained results in this paper from the investigation the effect of Mg\(^{2+}\) ion on Staphylococcus aureus-DNA Ligases enzyme type A (SLE-A) activity showed that 500 µM of Mg\(^{2+}\) ion had the best on ligases’s activity for fixing the nick in the DNA between the 20 mer and 30 mer, and transfer them to 50 mer. Comparing this result with different study by using ammonium sulphate ion to determine how much this ion affected to the E. coli DNA Ligase activity (LigA), and the result was showed that the best concentration of ammonium sulphate was as well 500 µM, which is indicated the similarity between these ions activity on different enzymes \(^{26}\). Moreover, the results can be concluded that DNA ligases of E. coli and Staphylococcus aureus are structurally similar. The \(K_m\) value (equilibrium association constant) in this study was 5.2 µM, which is explained the binding affinity between DNA ligase of Staphylococcus aureus SLE-A and MgCl\(_2\) ion was slightly similar to the \(K_m\) value for NAD\(^+\) cofactor for S. aureus LigA (2.75 µM) that found by Gul et al, 2004 \(^{27}\). However, different result by Sriskanda & Shuman 2001 who obtained the \(K_m\) value for E. coli DNA ligase enzyme LigA for singly-nicked DNA substrate and was 12.2 nM, which is completely different comparing with this study \(^{7}\). Generally, it was mentioned above in the discussion that the main objective in the biology and medical sciences is to find a target in the bacteria to be as novel drug target for antimicrobial therapy, and DNA ligase is one of them since there are no similarity to the eukaryotic DNA ligases. However, magnesium ions here did not inhibit the activity of Staphylococcus aureus-DNA Ligases enzyme type A (SLE-A), but increase the rat of reaction. Therefore, Due to the importance of the DNA ligase enzyme, further study would be involved to identify more compounds that would be able to inhibit the activity of DNA ligase (SEL-A) but not inhibit the activity of eukaryotic DNA ligases by blocking the communication sites between the
magnesium ions (Mg$^{2+}$) and the ligase of bacteria, and eventually control the growth of them.

**Acknowledgment:** The authors are very grateful to the University of Mosul for providing assistance and facilities which allowed finishing this work properly, with many thanks to the College of Agriculture and Forestry, College of Environmental Science and Technology and College of Education for Pure Science for kind support.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Study of Bacterial Contaminants and some Chemical Elements of the Euphrates River and Al-Fallujah City Drainage

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Abstract
This article included bacteriological and some chemical elements study of pollution from Euphrates river and Al-Flaloha city drainage. The study of four selected sites which are Euphrates river (T), Al-Falahat (F) drainage, Al-Ne'emya (N) drainage, and Al-Bo Alwan (B) drainage in the period 11-13/December/2016. Bacteria were isolated and diagnosed in water samples depending on their cultural, microscopic biochemical tests. Results showed that the water samples were polluted with bacteria produced by human and animal wastes, 7 out 20 isolates were selected and diagnosed. The study included counting the concentration of some chemical ions such as Sodium, Potassium using a flame photometer, the highest concentration of Sodium was in station F and the highest concentration of Potassium was at the station B, concentrations of carbonates and bicarbonates were measured using titration method, the highest concentration of bicarbonates ion was at station T, it also showed no carbonates in a water sample.

Keywords: Bacterial pollutants, Chemical elements, Euphrates, bacteriological, Bacterial isolates

Introduction
The considerable prevalence of salts in the agricultural lands located between the Dijla and Euphrates rivers in the plain sedimentary region led to the necessity of establishing a main network of puncture for the country’s need for fertile agricultural lands. Still the problem of biological pollution that is caused by sewage water is one of the most critical problems that have been aggravated by the political conditions, it went through such as wars, blockades and others that have increased water treatment problems. The current reality of treatment stations is one of the most important environmental problems that will undoubtedly lead to pollution of wastes, and of drains and hence the pollution of water bodies. The severity of water pollution is closely related to the source of pollution, and factory water and wastes constitute (60%) of all water pollutants, most of these pollutants are emitted from factories and their chemical waste, containing chemical elements pollution water. This makes this water a source of pathogenic bacteria such as Faecal Coliform Bacteria, Enterococcus and Pseudomonas Bacteria, which are evidence of stool water pollution.

In light of this: We use this study to detect the degree of pollution present in the studied water stations. As well as the diagnosis of bacteria resulting from pollutants and wastewater using microscopic and chemical analyzes.

Materials and Methods
The Study Area
Samples were collected from the study sites by one sample from each region during a season in addition to the sample taken from the Euphrates River using containers of polyethylene (1 liter) as shown in Table 1.
Identification of isolates

Microscopic and biochemical tests were performed using the scientific sources used in the diagnosis of bacteria. Diagnostic steps included cultural properties, Microscopic tests, and motility tests. As for biochemical tests, they included conducting the Oxidase test, the Catalase test, and the Citrate utilization test, Voges-Proskauer test, Methyl red test and Indole production test.

Determination of chemical elements in water samples

Determine the potassium $K^+$ and sodium $Na^+$ elements by flame atomic emission using a flame photometer and prepare a standard curve for the potassium element from KCl and a standard sodium curve from NaCl, the carbonate ions ($CO_3^{2-}$) and the bicarbonate ($HCO_3^{-}$) were measured by solution ($0.01 n H_2SO_4$).

Results and Discussion

The diagnosis was performed on (20) isolates obtained from the water samples of the studied sites according Bacterial Density, by (3 isolates at site T, 4 isolates at site F, 9 isolates at site N and 4 isolates at site B).

Micro and agricultural characteristics

Microscopic examination showed that the isolates that we obtained were (17) isolates in single or bilateral bacilli and (3) isolates in the form of staphylococci. As for the dye of gram, there was (15) negative isolation of gram. And (5) isolation is positive for gram. As for its ability to move, there are (14) of them grown on the medium semi-solid agar and (6) non-move isolates. Most bacteria isolates showed that ability to grow at a temperature of (28$^\circ$) and (38$^\circ$), as shown in Table No. (1).

Identification of isolates

<table>
<thead>
<tr>
<th>Site number</th>
<th>Location</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>site No. 1</td>
<td>Euphrates River in the city of Fallujah</td>
<td>T</td>
</tr>
<tr>
<td>site No. 2</td>
<td>AL-Flfalhat trocar In AL-Flfalhat suburb</td>
<td>F</td>
</tr>
<tr>
<td>site No. 3</td>
<td>AL-Nuaimia trocar in AL-Nuaimiya suburb</td>
<td>N</td>
</tr>
<tr>
<td>site No. 4</td>
<td>AL-Baalwan trocar in AL-Baalwan suburb</td>
<td>B</td>
</tr>
</tbody>
</table>

Biochemical tests

The results of the biochemical tests showed that the isolated isolates were tested for the tests (catalyze and oxidase enzyme production, indole production, red methyl test, fox proscur and the consumption of $Citrate$) as follows:

The catalase enzyme test

The catalase enzyme test showed that for all isolates there are 17 positive isolates of this test, that is, they have the ability to produce the catalase enzyme, and 3 negative isolates for this test that is, they cannot produce the catalase enzyme.

Oxidase test

The test showed that for all isolates there are 3 positive isolates that have the ability to excrete the oxidase and 17 negative isolates for this test, they do not have the ability to excrete the oxidase.

Indole production test

The indole production test for all isolates showed that there are 7 isolates on the ability to produce indole from the amino acid Tryptophan by enzyme Tryptophanase and 13 isolates that do not have the ability to produce indole.

Red Excitation Test

For all isolates, a red methylation test showed that 13 isolates showed red color. This indicates the
efficiency of isolated bacteria on analyzing glucose sugar altogether and produced amino mixed acid fermentation. And 7 isolates in which a yellow color was observed, this indicates the negativity of these isolates for this test, that is, they do not have the ability to thorough analyze glucose and produce this Mixed Acid Fermentation completely).

**Fox Proscower Test**

The results of the Fox Proscur test showed that for all isolates, there are 9 isolates that showed a red color, and this indicates the ability of bacteria to partially analyze glucose sugar and the formation of an intermediate compound is Acetyl-Methylcarbinol and 11 isolates in which a yellow color remains, and it has no ability to partially analyze glucose and form the intermediate Acetyl-Methylcarbinol.

**Citrate Utilization Test**

The test showed the utilization of *citrate* for all isolates that 9 isolates are changing the color The proof blue bromothaimol from green to blue this is evidence of the ability of bacteria in these isolates to use the *citrate* as a single source of carbon and the presence of the enzyme Citatelyase and 11 isolates where it was observed that the color of the green medium was not changed, evidence for this indicates that this isolation is negative for this test.

**Spread Bacteria in the studied areas:**

The areas that were studied trocar showed that there are a large number of different pollutants in them, especially their pollution sewage, agricultural wastes and animal waste, in addition to their pollution with factory waste, which caused an increase in the number of bacteria in those areas, as shown in Table (2) as follows:

### Table No. (2) bacterial genera isolated from the water of the Euphrates River and the ditches of the city of AL-Fallujah

<table>
<thead>
<tr>
<th>Bacterial Genera</th>
<th>Number of isolates</th>
<th>Isolation%</th>
<th>T</th>
<th>F</th>
<th>N</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>/</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>35</td>
<td>/</td>
<td>/</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>3</td>
<td>15</td>
<td>/</td>
<td>2</td>
<td>/</td>
<td>1</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>/</td>
<td>1</td>
<td>/</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>2</td>
<td>10</td>
<td>/</td>
<td>1</td>
<td>1</td>
<td>/</td>
</tr>
<tr>
<td>Enterobactrium</td>
<td>2</td>
<td>10</td>
<td>/</td>
<td>/</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>total number</td>
<td>20</td>
<td>100</td>
<td>3</td>
<td>4</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>
We notice from the results in Table that the *E. coli* bacteria have the largest number of bacterial isolates diagnosed by seven isolates and at a rate of (35%), as station N recorded the highest number of isolates for this bacterium by six isolates followed by station B with only one isolate. The reason for its spread in these areas may be due to the large pollution of these trocars with heavy water resulting from the disposal of wastewater, as well as throwing agricultural and animal wastes directly into the trocars, especially site N, as it is considered an agricultural area there is also a lot of animal husbandry in this region, as the high number of colon bacteria in the water is considered evidence of pollution of water with stool, which may be caused by drainage of wastewater into surface waters such as rivers, streams, etc. In addition to throwing agricultural wastes and animal waste in this water, which provides a fertile environment for the growth of this type of bacteria.

As for *P. aeruginosa* and *E. faecalis*, it was 15% each, and by one or two isolates of the station, the presence of these two types of bacteria is also an important indicator of bacteriological pollution of water resulting from human excrement and excreta in addition to other wastes. While bacterial species *Pr. mirabilis*, *K. pneumonia* and *Enterobactrum* are 10% each, and the presence of these bacterial species in the water is considered a danger to human health if this water is used for drinking, as *Klebsiella Pneumonia* is one of the causes of bowel disorders and pharyngitis, as well as urinary tract infection that may cause it. Also, *Enterobactum bacteria*

As for the bacteria *Citrobacter freundii*, the lowest percentage of bacterial isolates (5%) was recorded with only one isolate at station T, as this station has the advantage of being the lowest number of bacterial isolates diagnosed in it because the river water is running water and heavy elements are deposited as well as the discharge of pollutants.

Content of some chemical elements in water samples:

Positive ions:

Sodium ion:

Sodium (Na) concentrations in water samples have been reported between 215.6 mg.l⁻¹ at site B and 762.5 mg.l⁻¹ at site F and 443.1 mg.l⁻¹ at site N, while its concentration in the Euphrates River (T) was 104.9 mg.l⁻¹ as shown in Table (3). When comparing the results of sodium concentration, it appears to us that the highest value of the Na concentration was at site F, This may be due to the drainage of agricultural lands on both sides of the trocar, as the Al-Falahat suburb is considered an agricultural area containing many palm groves and other trees, where the process of trocars and evaporation in summer causes an increase in the percentage of salts in the water, including sodium salts. Whereas, the lowest value of the sodium concentration was recorded in the site T, because the river water is running water in which the concentration of chemical elements decreases in general, and also because of the growth of algae, and living organisms that consumer these nutrients.

Potassium ion:

The results of the study showed that the highest value of potassium ion concentration was for site B 16.7 mg / l, followed by site F 8 mg / l, then site N 4.8 mg / l. As it is noticed the low potassium concentration in the water compared to the sodium ion, This is due to the element potassium that is included in the composition of clay minerals during weathering and the other reason is that potassium minerals are more resistant to weathering compared to sodium minerals. While the lowest value of the Euphrates River T was recorded at 4.4 mg.l⁻¹, because the river water is considered running water and the consumption of the elements by the aquatic organisms that are present in it occurs a lot compared to other stations (Table No. 3).

Negative ions:

Carbonate ion, CO₃⁻ and bicarbonate, HCO₃⁻:

The results showed that the highest concentration of the bicarbonate ion was in the water of the river and this is consistent with the study carried out by, which also showed an increase in the concentration of bicarbonate ion in the Euphrates River for the city of Fallujah. The high concentration of these ions in the water of the river is due to the availability of dissolved non-organic carbon in addition to the heavy rains falling on the soil rich in carbon dioxide whose waters descend into the river, which is one of the main reasons leading to an
increase in the value of the bicarbonate ion \(^1\). As for other sites, we note that the site N is 6 mg.l\(^{-1}\), followed by the Euphrates River, followed by the site F 2 mg.l\(^{-1}\), then the site B is 0.9 mg.l\(^{-1}\). This is proportional to the number of diagnosed bacteria in these sites that rely on citrate as a carbon source, where it was observed that the concentration of the bicarbonate ion increased with the increase in the number of diagnosed bacteria in that water except for the Euphrates River water T 28 mg.l\(^{-1}\) where the concentration of the bicarbonate ion increased for the reasons mentioned above (Table No. 3). It is clear to us through the above results that there is an increase in the concentration of the chemical elements studied in the water stations included in the study, Explain \(^2\) indicated that sanitary and industrial wastewater always leads to a large increase in the concentration of chemical elements in the water.

Table (3) Concentration of chemical elements studied in water samples.

<table>
<thead>
<tr>
<th>Co3 Concentration mmol / L</th>
<th>HCO3 concentration mmol / L</th>
<th>Concentration + K mg.l(^{-1})</th>
<th>Na + mg.l(^{-1}) concentration</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>28</td>
<td>4.4</td>
<td>104.9</td>
<td>T</td>
</tr>
<tr>
<td>Nil</td>
<td>0.9</td>
<td>15.7</td>
<td>215.6</td>
<td>B</td>
</tr>
<tr>
<td>Nil</td>
<td>2</td>
<td>8</td>
<td>762.5</td>
<td>F</td>
</tr>
<tr>
<td>Nil</td>
<td>6</td>
<td>4.8</td>
<td>443.1</td>
<td>N</td>
</tr>
</tbody>
</table>

Conclusions

Through the results of this study it was found that studied water suffers from the pollution that reached a degree that negatively affected its use; Hence, it became unsuitable for drinking and industrial home use, which affects public health. There was also a relationship between the pollution of this water and the increased prevalence of pathogenic bacteria in the areas under study. It also increases the concentration of elements in water samples due to their pollution with wastewater.

Ethical Clearance: Ethical clearance from the Ethical committee University of Anbar.

Conflict of Interest: Nil

Source of Funding: Self funding.

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Study of some Immunological Variables Resulted from Balb/c Mice Injection with Hydatid Cysts Protoscolex Antigens

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Abstract

Human cyst disease is a common epidemic disease between humans. The current study was carried out to study some of immunological parameters (the immunoglobins IgG, IgA, the complement proteins C3, C4, the MIF of PMNs cells, the reducing of NBT and the phagocytosis of Candida albicans) in the Balb/c mice injected with antigen of protoscolex. By using (15) BALb/c mice and the antibodies IgG, IgA in addition to the complements (C3, C4) that were measured as well as the Migration Inhibition Factor (MIF). The results showed that there were significant differences in IgG (13458) mg/dl. IgA (4773) mg/dl, C3 (359.7) mg/dl, C4 (90.80) mg/dl levels with injection of 0.5 ml in muscle and the MIF levels (1.49) with injection of (0.75) ml. so between the test and the control up (p≤0.05). This might be a good candidate for immunization and diagnosis of Hydatid cysts in the intermediate host of E. granulosus.

Keywords: Complement, Echinococcus granulosus, Hydatid cysts, Immunoglobulins, Protoscolex antigen

Introduction

Human cyst disease is a common epidemic disease between humans and animals since ancient times; this disease was caused by the larval stage of granular cestodes of the genus E. granulosus ¹⁻⁵. The lifecycle of this parasite requires two hosts, the final host, such as dogs, which carry adult worms, and the central host, such as humans, who infected with the larval phase that cause water cyst disease. The disease is endemic in southern America, northern Canada, Western Europe, the Mediterranean basin and the Asia center, and in Australia ⁶⁻⁸. The Hydatid cysts infect the organs and tissues of the body ⁹⁻¹¹ and liver infection represent more than half of the organs’ infections¹². While spinal cord and brain injuries are very rare¹³, the bone injury was first described by Didlon in 1870. ¹⁴ The survival of the parasite in the body may extend for 53 years ¹⁵ and this long survival may explain its ability to regulate the immunological response ¹⁶.

Therefore, current studies have focused on knowing how to stimulate the immune system in the central host and the possibility of using its Antigens of whole-numerical components. Therefore, the present study focused on the possibility of preparing antigens from protoscolex proteins and using different concentrations of these antigens in order to stimulate the immune response in laboratory animals by immunization of the mice with protoscolex antigens using two concentrations intramuscular.

Material and Methods

Experimental design :

1. The experiment dealt with 15 white mice distributed into three groups of 5 mice each.

2. The first group was injected with a concentration of 0.5 ml of protoscolex antigen I/M. The second group was injected at a concentration of 0.75 ml of the same antigen. The third group injected at a concentration of 0.1 ml PBS solution as a control group at the same location.

3. Measurement the level of serum IgG, IgA in immunized mice using single radial immunodiffusion
Measurement of neutrophil PMNs and Testing the Phagocytosis of *Candida albicans* by dye exclusion method according to 17.

4. Measurement of NBT dye reductively according to 18.

5. Measurement Coefficient of inhibition of cell migration and Complement measurement using proliferation of a single immune protein in solid pits.

Statistical analysis: mean and standard deviation calculated using SPSS program.

Results and Discussion

The results of the study revealed a significant increase in IgG and IgA antibody production levels in the plentiful serum of mice. The highest concentration of IgG and IgA were recorded respectively (4773) and (3458) mg / 100 ml at antigen concentration 0.5 ml compared to control.

Table (1) Serum levels of IgG and IgA in mice immunized with hydatid cyst protoscolex antigens

<table>
<thead>
<tr>
<th>Antigen Concentration</th>
<th>Serum levels of IgG and IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG mg/ 100ml</td>
</tr>
<tr>
<td>0.5</td>
<td>5.3458.2 ± 280</td>
</tr>
<tr>
<td>0.75</td>
<td>3327.8 ± 166.6</td>
</tr>
<tr>
<td>PBS</td>
<td>1013.3 ± 0.87</td>
</tr>
</tbody>
</table>

Concentration of complement proteins C4, C3 mg/100 ml. in white mice immunized with hydatid cyst protoscolex antigen

Significant increase appeared in the production levels of C3 - C4 passage proteins in the antigen – immunized group using 0.5ml compared to the control group, where the study recorded a consecutive concentration (90.80) and (359.7) mg/100 ml compared to 0.75 ml of antigen ,where the concentration were 70.98 ± 0.36 and 322.8 ± 26.67 respectively as indicated in Table 2.

Table (2) complement proteins concentration in mice immunized with two different dose of hydatid cyst protoscolex antigen

<table>
<thead>
<tr>
<th>Antigen Concentration</th>
<th>Complement proteins Concentration rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4 Concentration rate (mg/ 100ml)</td>
</tr>
<tr>
<td></td>
<td>±SD</td>
</tr>
<tr>
<td>0.5</td>
<td>90.80 ± 2.79</td>
</tr>
<tr>
<td>0.75</td>
<td>70.98 ± 0.36</td>
</tr>
<tr>
<td>PBS</td>
<td>55.68 ± 1.13</td>
</tr>
</tbody>
</table>

NBT reduction results indicate the presence of significant differences between two different doses of antigens in mice. And it reached the highest 22.74 ± 2.17 in 0.5ml and 0.75 ml of the antigen compared with the control (15.29%). (Table 3)
Table (3) indicates the effect of immunization of white mice with two doses of protoscolex antigen on NBT reductions.

<table>
<thead>
<tr>
<th>Antigen Concentration</th>
<th>NBT reduction percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>22.74 ± 2.17</td>
</tr>
<tr>
<td>0.75</td>
<td>23.38 ± 3.73</td>
</tr>
<tr>
<td>PBS</td>
<td>15.29 ± 0.83</td>
</tr>
</tbody>
</table>

Figure (1) the Reduction of NBT by protoscolex antigen of hydatid cyst.

The results showed significant differences in the Migration Inhibition Factor (MIF) for the two concentrations of the antigen in the muscle compared control as showed in Table (4)

Table (4) MIF test in immunized mice with hydatid cyst antigen under p≤0.05.

<table>
<thead>
<tr>
<th>Antigen Concentration</th>
<th>MIF rates in mm</th>
<th>MIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>22.29 ± 0.72</td>
<td>1.47 A</td>
</tr>
<tr>
<td>0.75</td>
<td>23.38 ± 3.73</td>
<td>1.49 A</td>
</tr>
<tr>
<td>PBS 0.1</td>
<td>15.29 ± 0.83</td>
<td>1 B</td>
</tr>
</tbody>
</table>

Figure (2) Migration Inhibition Factor under effect of Protoscolexx antigen.
Effect of hydatid cyst protoscolex antigen immunization of white mice with two doses of protoscolex antigen on phagocytosis coefficient of C. albicans.

There were no significant differences between of all groups at all times and the highest percentage was (84.33%) in 30 minutes compared to the control group (80.48) as indicated in Table (5).

Table (5) Effect of immunization of white mice with two doses of primary antigenic antigen on Candida albicans yeast.

<table>
<thead>
<tr>
<th>Antigen concentration</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>83.21± 1.41</td>
<td>84.25± 1.41</td>
<td>83.21± 1.54</td>
<td>83.31± 1.22</td>
</tr>
<tr>
<td>0.75</td>
<td>84.33± 0.58</td>
<td>81.36± 1.24</td>
<td>83.59± 1.15</td>
<td>70.94± 2.87</td>
</tr>
<tr>
<td>PBS</td>
<td>80.48± 0.81</td>
<td>81.44± 1.06</td>
<td>83.25± 1.55</td>
<td>77.08± 0.61</td>
</tr>
</tbody>
</table>

Protoscolex antigens are highly effective in stimulating and activating the immune system, especially in non-lethal doses so that non-expansion of both liver and spleen resulted from the determination of the disease by the immune response of the host, where the spleen is the most resistant organ because it is an immune organ that includes cells that regulate the immune response. The immune response against cystic adenocarcinoma involves the production of fixed levels of antibodies, but these levels are rapidly reduced after removal of the cyst and its organs. IgG levels rise within 2 to 11 weeks of infection. Where the antibodies act in killing the protoscolex of E. granulosus worms in the chronic stages of infection, mainly IgG, IgM IgA, antibodies. These levels begin to decline in the chronic stages of the infection but start to rise in the later stages. Where levels of IgG1, IgG3 significantly increased during the eighth week after the challenge dose and subsequent periods. The immune response mediated by complement and gastric proteins by bodies Antibiotics can be generated by administering two doses of the vaccine within a month and lasting up to 13 months. There is evidence that a significant increasing in PMNs and macrophage and eosinophils. Some cytokines that are secreted by lymphocytes type - T2 cells can inhibit the effectiveness of the influencing factors despite the stimulation of large numbers of immune cells.

The production of IFN gamma, IFN alpha, interleukins when stimulating cells with antigens plays an important role in the secondary response. Those materials work in inducing T-independent antigens, which in turn stimulate the production of antibodies type IgG3 IgM as there is a clear link between the production of antibodies and the production of cytokines in the chronic stages of infection in humans in IgG4 and IgE types. This binding is necessary to determine the growth of the parasite and prevent the destruction of the organ in the host. The ability of cells to phagocyte germs and antigens depends on their ability to produce super oxide ion, which plays a key role in the mechanism of cell killing, gobbling and destroying antigens when assembling H₂O₂, O₂. However, they do not stimulate lymphocytes qualitatively, but they do not produce sufficient amounts of MIF.

Conclusions

Finding of this research support involvement of immune system competent in mice vaccinate with E. granulosus antigens, and in the sequential promotion of the immune cell responses and a modulate immunity in infective animals to moderate vaccines.

Source of Funding- Self

Conflict of Interest- Nil.

Ethical Clearance: Taken from the Scientific Committee College of Veterinary Medicine, University of Fallujah.
References:


Study of the Biochemical Markers of Liver and Renal Function in Moderate -To- Heavy Cigarette Smokers’ Men in Mosul City

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Abstract

Smoking has been associated with adverse health effects on body organs. The effect of smoking on the liver’s enzymes and kidney’s parameters, and their relationships with some blood parameters need more evaluation. This study aimed to investigate the association of cigarette smoking to liver and kidney functions in 68 males, all of them not suffer from chronic disease and their ages were between (25-45) years, by estimating the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as liver enzymes as well as estimating of creatinine, urea, and uric acid in the serum of smokers comparing with non-smokers groups (control). Also, this work aimed to study the effects of smoking on some blood profiles, white blood cell counts (WBC), red blood counts (RBC), hemoglobin (HB), mean corpuscular volume (MCV), and platelet (PLT). The results showed a significant increase in (ALT), (AST), (ALP) enzymes, urea, creatinine (WBC), (RBC), (HB) and (MCV), the result also showed a significant decrease in uric acid while no significant decrease in (PLT) in smokers comparing to non-smokers’ group. In conclusion a cigarette smoking negatively affected in biochemical markers of liver and kidney function.

Keywords: Non-smokers, biochemical markers, liver, renal.

Introduction

Smoking is one of the main causes of disability, illness and early death 1. The use of tobacco is widely spreading throughout the world and was listed as one of the risk factors for the majority of the disorders. Different diseases are caused or gotten worse by cigarette smoking that leads to death which is appearing every year all over the world 2. Recent investigation by Gue 3 suggests that active smoking is significantly related to the risks of the severe cases of COVID-19. The death that is caused by heart disease was strongly related to the lower levels of education 4. However, alcohol, smoking, body mass index, and physical inactivity were considered as most of the risk factors of atherosclerosis and cardiovascular disease, and the biggest share was accounted for smoking 4, 5. The good news by Babb et al. was 68% of cigarette smokers want completely to quit smoking 6.

The best define of moderate-to-heavy smokers is the persons who are smoking at least 11 cigarettes per day 7. Cigarette Smoking induces major adverse effects and exposes the body to many different harmful compounds such as toxins, free radicals, and carcinogenic 7. These compounds have numerous impacts on diverse body organs starting with the primary contact of cigarette poisons to the lung and other organs 8. Although cigarette smoking is the main risk for cardiovascular diseases, its relationship with hypertension remains unclear 9.

Smoking is one of the poor living behavior in our life, that may elevate the risk for liver disease such as cirrhosis and fibrosis that associated with chronic hepatitis B infection 10, 11. Azzalini et al. have mentioned a relationship between the seriousness of the liver infection and cigarette smoking in patients with liver disease 12. The liver is the important organs that is negatively affected by smoking. The liver plays the main role of metabolism, storing the Glycogen, and eliminating the harmful and toxic compounds from the body 13.

In addition, the kidneys control the consistency of the inner environment, valuable materials are rapidly recycled but undesirable ingredients are effectively eliminated through urine 14. A positive relationship
between smoking and chronic kidney disease (CKD) has been mentioned. Since there is a correlation between the function of the liver and the kidney, it is possible that liver disease in more than one way affects the functioning of the kidney and increases the rates of microbial infections. Also, the decrease in blood pressure resulting from a defect in the function of the liver leads to a decrease in blood flow that reaches the kidneys and it becomes unable to perform its function in eliminating toxins from the body, which causes an increase in the level of creatinine and urea in the blood.

More clarification might be required about the effect of smoking on liver and kidney functions. This study aimed to investigate the effect of cigarette smoking on liver function, kidney function, and blood profile. In addition, this study interested in evaluating the possible associations between the liver’s enzymes and parameters studied such as urea, creatinine, uric acid, and some of the blood parameters. Experimental section:

**Materials and Methods**

A total of (68) Iraqi males in Mosul city were separated into two groups: (34) smoker and (34) non-smokers (control), their ages were between 25-45 years. All participants did not suffer from any chronic diseases and did not take drugs. Each person included in this study was asked about the duration of smoking and also the number of cigarettes consumed per day.

A blood sample was collected from the vein, a small amount of each sample was separated in the test tube that contains anticoagulant with gentle mixing for the use in CBC test, and the rest was left to clot in Serum Separation Gel Tubes, sera were stored in labeled Eppendorf tubes at -20°C until they were used for the biochemical test.

Body mass index (BMI) value was estimated by the weight in kg per the body height square in meters (kg m−2). (ALT), (AST) and (ALP) activities in serum were estimated by using the Kits from Biolabo-amazing Company, made in France. Urea concentrations in sera were estimated by using Kits from BioSystems company, made in Spain. Serum creatinine and uric acid concentrations were estimated by using Kits from Biolabo-maize-France.

**Statistical measurements:**

By using SPSS (Statistical Package for the Social Sciences) software program version 26, data of this study were analyzed. The normality of data was tested by Kolmogorov-Smirnov test. Independent-Sample T-test was performed for the comparisons between two groups (Smokers and control). Bivariate Analysis (person correlation for normal distribution data and Spearman correlation for abnormal distribution data) was performed to find possible associations between liver enzymes and the selected parameters in the smokers group. P-value ≤0.05 is considered significant.

**Results and Discussion**

The effects of Smoking depend on the duration of smoking and how much the individual smokes per day. Early smoking increases the risk factor of linked diseases. The characteristics of the subjects that included Ages, BMI, and duration of smoking in both non-smokers (control) and smokers’ group were shown in Table 1.

### Table 1 Characteristics of the subjects (smokers and non-smokers’ group), Data expressed as Mean ± SD

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-smokers</th>
<th>Smokers</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 34</td>
<td>No. 34</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.52 ± 6.86</td>
<td>34.29 ± 6.88</td>
<td>25 - 45</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>26.79 ± 2.71</td>
<td>25.22 ± 2.61</td>
<td>18.4- 28.2</td>
</tr>
<tr>
<td>Duration of smoking (years)</td>
<td>-----------</td>
<td>9.18 ± 3.6</td>
<td>5 – 16</td>
</tr>
<tr>
<td>No. of cigarettes consumed by smokers/day</td>
<td>-----------</td>
<td>22.23 ± 6.63</td>
<td>15 – 35</td>
</tr>
</tbody>
</table>
BMI, body mass index

The liver is an active organ with various functions, such as regulating glycogen storage, production of plasma protein, and detoxification. The results showed that enzyme activity of ALT, AST, and ALP in serum increased significantly in cigarette smokers in comparison with non-smokers as seen in Table 2. This may be indicating the negative effect of smoking on the performance of the hepatocyte. These results were similar to the results of Gordon. Numerous studies suggest that AST, ALT enzymes can be used as markers for any disorder related to the function of the liver, and as indicators of clinical results for the patients and healthy groups. The high levels of liver enzymes ALT, AST, and ALP in the smokers’ men may be due to the release of more than normal levels of the cellular oxidative radicals. This effect may be because there are many different types of components in cigarette smoke able to disrupt the balance of oxidants and antioxidants in all tissues and blood. Cigarette smoke influenced liver function by harmful and toxic compounds on hepatocyte, that lead to secrete enzymes from the cells of the liver through inflammatory pathways, and that increases the chronic inflammation. There is a multivariable that affects the serum levels of ALT and AST such as BMI, age, gender, daily current smoking, and lifetime of smoking.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-smokers Control No. = 34</th>
<th>Smokers No. = 34</th>
<th>P_value</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT IU/L</td>
<td>11.2 ±1.67</td>
<td>21.44 ± 2.59 *</td>
<td>&lt;0.0001</td>
<td>0 – 45</td>
</tr>
<tr>
<td>AST IU/L</td>
<td>10.3 ±1.56</td>
<td>17.6 ±1.8 *</td>
<td>&lt;0.0001</td>
<td>0 - 35</td>
</tr>
<tr>
<td>ALP IU/L</td>
<td>67.4± 11.1</td>
<td>77.7 ± 14.4 *</td>
<td>0.002</td>
<td>30 – 120</td>
</tr>
<tr>
<td>urea mg/dl</td>
<td>20.1 ± 3.2</td>
<td>25.1 ± 3.37 *</td>
<td>&lt;0.0001</td>
<td>7- 20</td>
</tr>
<tr>
<td>creatinine mg/dl</td>
<td>0.849 ± 0.09</td>
<td>1.1 ± 0.16 *</td>
<td>&lt;0.0001</td>
<td>0.84 -1.44</td>
</tr>
<tr>
<td>uric acid mg/dl</td>
<td>4.81 ± 0.65</td>
<td>3.8 ± 0.54 *</td>
<td>&lt;0.0001</td>
<td>3.4 - 7</td>
</tr>
</tbody>
</table>

ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase. * significant at p-value ≤0.05

This study also showed the alterations in the levels of creatinine, urea, and uric acid in the smokers’ group in comparison with the control that obviously appears the negative effect of smoking on kidney function. Table 2 shows significantly elevated levels of serum urea and creatinine in smokers than in the control. These findings have similarity with the outcomes of another investigation by El Sayed et al. The cause of these changes might be due to a rise in resistance in renal vascular, leading to lowering in the rate of glomerular filtration, a decrease in the rate flow of distal renal tubular, and a fall of urea reabsorption. The result also showed a significant decrease in serum uric acid concentration in smokers in compression with nonsmokers’ groups. This result matches with other reports by Gorica and Skibska, that indicated low serum uric acid in the smokers’ groups. The decline or destruction of the antioxidants levels, indicating that the oxidative stress is increased with every cigarette smoked as demonstrated. Cigarette smoking influences numerous organs of human body. It causes vasoconstriction due to stimulation of the nervous system (sympathetic) which leads to the rise of blood pressure and reduction in glomerular filtration rate. Also increases the risk of microalbuminuria.
Moderate-to-heavy daily cigarette smokers showed a significant increase in WBC compared with the control who had normal value for the above parameters as seen in Table 3. This result is similar to the results of Freedman et al. 28.

### Table 3 Effect of smoking on blood parameters. Data expressed as Mean ± SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-smokers’ group No.=34</th>
<th>Smokers’ group No.=34</th>
<th>p-value</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^9/L)</td>
<td>4.5 ± 0.73</td>
<td>6.8 ± 0.94 *</td>
<td>&lt;0.0001</td>
<td>3.5 – 9.6</td>
</tr>
<tr>
<td>RBC (10^12/L)</td>
<td>4.8 ± 0.5</td>
<td>5.77 ± 0.75 *</td>
<td>&lt;0.0001</td>
<td>3.1 - 4.8</td>
</tr>
<tr>
<td>HB (g/dL)</td>
<td>15.4 ± 1.1</td>
<td>16.5 ± 1.2 *</td>
<td>&lt;0.001</td>
<td>11.1- 14.8</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>86.9 ± 4.3</td>
<td>91.6 ± 5.8 *</td>
<td>&lt;0.001</td>
<td>83.3 - 98</td>
</tr>
<tr>
<td>PLT (10^9/L)</td>
<td>203.9 ± 21.2</td>
<td>197.4 ± 17.4</td>
<td>0.16</td>
<td>159 -367</td>
</tr>
</tbody>
</table>

WBC; white blood cell, RBC; red blood cell, HB, hemoglobin, MCV; Mean corpuscular volume,

PLT; platelet blood count. * Significant at p-value ≤0.05

It is known that the high levels of leukocytosis in smokers’ blood could be a marker of the injury or inflammation in the tissues due to high level of free radicals as oxidants in cigarette smoke 29. In addition, smoking leads to an increase in the WBC level by 30% in comparison with non-smokers. Glycoprotein as ingredients of tobacco leaf might play a role as a stimulator for the lymphocyte to activate proliferation and differentiation through its interaction with membrane components, inducing response such as the response that occurs with antigen. This mechanism could be one of the possible mechanisms that may cause the elevation in the WBC account as demonstrated 28. RBC, HB and MCV also increased significantly in the smokers’ group compared with control, as shown in table 3, this elevation because of the presence of Carbon monoxide, one of the chemical compounds recognized in tobacco that may be lead to hypoxia and then the body increases the number of erythrocytes due to hypoxia 30. This situation of polycythemia creates an oxy-carbon poisoning, so more oxygen is required and the generation of RBC cells is increased 31,32. MCV is one index of the RBC that help to measure the HB composition and average size of the RBC. The result showed no significant decrease in platelet (PLT) count in the smokers’ group compared with the control as seen in table 3. The current study were slightly showed in agreement with another study that found that there was no significant change between male smokers and nonsmokers in the levels of the platelet counts 33. Another study by Suwansaksri and Wiwanitkit observed there was no difference in platelet levels in male smokers and control 34. However, the effect of smoking on the amounts of platelets might be another contributing factor and it is still controversial. A higher level of hemoglobin as a result of decreasing the proportion of oxygenated blood and increasing the carboxyhemoglobin level in the blood leaves no place on the hemoglobin in the RBC to carry oxygen. Hypoxia induces the secretion of the erythropoietin hormone from the kidneys which activates the bone marrow to increase red blood cells’ production in order to overcome this deficiency. In addition, smoking causes lipoprotein abnormalities that have been detected in active smokers. As a symptom of these changes, the smoker feels tired when he is doing the simplest physical activities as demonstrated by Gossett and his workers 35. Bivariate analysis was performed to find possibly related between the liver’s enzymes and the selected parameters in the smokers’ groups. The results showed there is no correlation with ALT enzyme and other
selected parameters as shown in Table 4. A negatively significant correlation between AST enzyme and ALP enzyme and a significant positive correlation between AST enzyme and uric acid showed in Table 5. However, there is no correlation between the ALP enzyme and the parameters studied as shown in Table 6. The elevation in ALP concentration is related to liver dysfunction and it is caused by some destruction of hepatic cell membranes such as intrahepatic or extrahepatic cholestasis. Rising cholestasis accelerates more production of ALP by the bile ductules cell producing an additional level of ALP which ends up entering circulating. ALP enzyme is present in the liver and bones, a high level of ALP in the blood without any increase in the liver enzymes may be good markers of a specific injury in the gall bladder tracts. Nyblom et al. found significant elevations of AST and plasma uric acid in patients with cirrhosis of the liver, viral hepatitis. The increase in serum uric acid concentration with the elevations in the liver enzymes levels might be accounted as a risk factor that related to chronic liver diseases.

Table 4 Correlations of alanine aminotransferase (ALT) with the selected parameters in smokers group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient</th>
<th>p-value; sig-(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>0.184</td>
<td>0.296 p</td>
</tr>
<tr>
<td>ALP</td>
<td>-0.174</td>
<td>0.325 p</td>
</tr>
<tr>
<td>Urea</td>
<td>-0.232</td>
<td>0.187 *</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.074</td>
<td>0.679 *</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0.123</td>
<td>0.487 p</td>
</tr>
<tr>
<td>WBC</td>
<td>0.312</td>
<td>0.072 p</td>
</tr>
<tr>
<td>RBC</td>
<td>0.278</td>
<td>0.112 p</td>
</tr>
<tr>
<td>HB</td>
<td>0.325</td>
<td>0.061 S</td>
</tr>
<tr>
<td>MCV</td>
<td>0.148</td>
<td>0.404 S</td>
</tr>
<tr>
<td>PLT</td>
<td>-0.286</td>
<td>0.101 P</td>
</tr>
</tbody>
</table>

; Pearson correlation, S; Spearman correlation, significant at p-value ≤0.05ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase. WBC; white blood cells, RBC; red blood cell, HB; hemoglobin, MCV; Mean corpuscular volume, PLT; platelet blood count.

Table 5 Correlations of aspartate aminotransferase (AST) with selected parameters in the smokers group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient</th>
<th>p-value; sig-(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>0.184</td>
<td>0.296 p</td>
</tr>
<tr>
<td>ALP</td>
<td>-0.341*</td>
<td>0.048 p</td>
</tr>
<tr>
<td>Urea</td>
<td>-0.335</td>
<td>0.053 S</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.271</td>
<td>0.122 S</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0.344*</td>
<td>0.046 P</td>
</tr>
<tr>
<td>WBC</td>
<td>0.273</td>
<td>0.118 P</td>
</tr>
<tr>
<td>RBC</td>
<td>-0.228</td>
<td>0.195 P</td>
</tr>
<tr>
<td>HB</td>
<td>0.009</td>
<td>0.962 S</td>
</tr>
<tr>
<td>MCV</td>
<td>0.146</td>
<td>0.411 S</td>
</tr>
<tr>
<td>PLT</td>
<td>0.22</td>
<td>0.903 P</td>
</tr>
</tbody>
</table>
Table 6 Correlation of alkaline phosphatase (ALP) with the selected parameters in the smokers group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient</th>
<th>p-value; sig-(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>0.296 p</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>0.296 p</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>0.184</td>
<td>0.387 S</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.184</td>
<td>0.302 S</td>
</tr>
<tr>
<td>-0.153</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0.013</td>
<td>0.942 P</td>
</tr>
<tr>
<td>WBC</td>
<td>-0.32</td>
<td>0.064 P</td>
</tr>
<tr>
<td>RBC</td>
<td>-0.089</td>
<td>0.25 P</td>
</tr>
<tr>
<td>HB</td>
<td>0.138</td>
<td>0.618 S</td>
</tr>
<tr>
<td>MCV</td>
<td>0.268</td>
<td>0.436 S</td>
</tr>
<tr>
<td>PLT</td>
<td></td>
<td>0.125 P</td>
</tr>
</tbody>
</table>

p; Pearson correlation, S; Spearman correlation, significant at p-value ≤0.05

Conclusions

Smoking is one of the great health risks that negatively affect the markers of liver and kidney functions and causes a significant increase in ALT, AST, ALP enzymes, urea, creatinine, WBC, RBC, HB, MCV and no significant decrease in PLT. Cigarette smoking significantly lower of uric acid. Decreasing serum uric acid significantly in smokers indicates that one or more of the cigarette smoking components might be useful for making this effect, but these compounds and the mechanism need to be clarifying by additional work.

Source of Funding-self

Conflict of Interest-Nil

Ethical Clearance- Taken from university of Mosul

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24. Mu, Y. Patters, B. J. Midde, N. M. He, H. Kumar, S. and Cory, T. J. “Tobacco and antiretrovirals modulate transporter, metabolic enzyme, and antioxidant enzyme expression and function in


The Effect of Dual-Species Biofilms, Monosaccharide and D-Amino Acids on Pseudomonal Biofilm

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¹Assistant lecture, University of Anbar, College of Education for Pure Sciences, Department of Biology, ²Assistant Professor, University of Anbar, College of Science, Department of Biotechnology, ³Professor, University of Anbar, College of Medicine, Department of Microbiology

Abstract

Background: The major therapeutic failure in clinical settings is due to problematic biofilm-producing bacteria like Pseudomonas aeruginosa. This study aims to investigate the effect of dual-species biofilms, monosaccharide, and D-amino acids on pseudomonal biofilm.

Methodology: A total of 130 patients with catheter-associated urinary tract and wound infections were involved in this study. Quantitative biofilm assay by alginate beads was performed. The dual-species biofilms have been done, and the effect of ciprofloxacin, monosaccharides, and D-amino acids on sessile cells was detected.

Conclusion: This study suggested that the combined action of both monosaccharides (glucose and galactose) and the combination of each one with ciprofloxacin is the enhancing of P. aeruginosa biofilm and increases survival strategy against ciprofloxacin. In contrast, a noticeable biofilm reduction and marked synergistic action for ciprofloxacin have been determined in the combination of the two D-amino acid, alanine, and glycine in comparison to the effect of each one alone. Furthermore, the dual biofilm of P. aeruginosa with each of K. pneumonia and E. coli, markedly reduced pseudomonal biofilm, while co-culture with S. aureus revealed strong support of pseudomonal pathogenicity and increased its biofilm production.

Keywords: Biofilm, monosaccharide, D-amino acids

Introduction

Pseudomonas aeruginosa, a Gram-negative bacillus widely found in nature, is an opportunistic pathogen that can cause disease in immunosuppressed patients.¹ The organism is known to produce biofilm and cause severe infections.² A biofilm is generally considered to be a community of microbes enmeshed in an extracellular matrix (often a polysaccharide), typically attached to surfaces, that displays characteristics different from their planktonic counterparts. These unique properties of biofilms complicate their eradication and may contribute to the development of chronic disease.³ Additionally, naturally occurring biofilms typically involve multiple species of bacteria. Polymicrobial growth necessitates interspecies interactions that involve some degree of intercellular communication and metabolic cooperation. The interactions within mixed-species biofilms have been characterized as cooperative, competitive, or neutral, nature based on the genetic background of the involved species.⁴

P. aeruginosa produces at least three extracellular polysaccharides with different chemical structures, functions, and biosynthetic pathways: alginate, Psl, and Pel. Pel is a glucose-rich structure and Psl consists of repeating D-glucose units. Some P. aeruginosa strains predominantly produce Psl, which is associated with matrix stability.⁵ Factors that can trigger biofilm dissolution of the biofilm are D-amino acids, which are naturally produced by late stationary phase cultures. Incorporating D-amino acids within the cell wall modulates peptidoglycan synthesis and inhibits attachment to the biofilm matrix proteins. Thus, these amino acids may reduce intercellular adhesion,
negatively affecting biofilm stability. Therefore, one of the aims of this study was to investigate the effect of monosaccharides and D-amino acids on pseudomonal biofilms, including their effects on biofilm inhibitory concentrations (BICs). Another aim of the study was to gain insights into inter-species interactions in dual species biofilms, such as survival strategies in the presence of antibiotics.

**Patients and Methods**

A total of 130 clinical specimens were obtained from patients with catheter-associated urinary tract and wound infections. The patients attended the Ramadi (Iraq) Teaching Hospital or private clinics in Ramadi between October 2019 and February 2020.

**Microbiological diagnosis of study isolates**

*P. aeruginosa* isolates were recovered following growth on selective media (2.2% nalidixic acid with cetrimide agar). All study isolates of *P. aeruginosa*, *S. aureus*, *K. pneumonia*, and *E. coli* were identified using the Vitek system (BioMérieux, Marcy-l’Etoile, France) and confirmed using biochemical tests.

**Detection of biofilm production**

Bacterial biofilms were produced on alginate beads, following the method of Vásquez-Ponce et al., Briefly, this method entails by cultured bacteria overnight in BHI broth, and added (600 µL) to the wells of a 48-well culture plate. Prepared alginate beads had been added to the wells and the plates were incubated overnight. The cultured beads were transferred to triplicate fresh wells containing BHI broth (600 µL) and the desired antibiotic, and the plates were incubated overnight, and then calculating by Mils Misra method.

Production was included in this study using alginate beads. It includes preparation of alginate beads, biofilm set up and formation on it, dislodging of sessile cells from beads and then counting bacteria by Miles Misra method the isolates’ ability to form biofilms was first determined by a qualitative microtiter plate assay. This test involved the inoculation in the wells of a 96-well microtiter plate of a standardized bacterial inoculum. The methanol was added to each well for 15 minutes after overnight incubation and then the plate was drained, allowing dry air to be allowed. Before the well was rinsed out, crystal violet for 5 min was added to each well. In each well, acetic acid (160 µl) was added and an optical density at 570 and 630 nm for each well was read. Optical (OD) density was used to classify the isolate’s capability to make a biofilm into weak (OD < 1.078), intermediate (OD = 1.078–2.156), or strong (OD > 2.156).

**Biofilm susceptibility test**

Susceptibility testing was performed according to the Clinical Laboratory Standard Institute (CLSI) method. Briefly, 600 µl of brain heart infusion (BHI) containing approximately 10⁴ colony forming units (CFU)/mL was pipetted into each well of a 48-well plate. Alginate beads, prepared as described by Allison et al., were also added (600 µL) to each well, and the plate was incubated (37°C, 150 rpm) overnight. Following the incubation, the media and non-adherent bacteria were removed and fresh media was added. In experiments investigating the effects of ciprofloxacin, the antibiotic was added to the media. For the experiments investigating the effects of added monosaccharides, the BHI broth was supplemented with glucose, fructose, or galactose at 20 µg/dL, or with combinations of glucose/fructose, glucose/galactose, or fructose/galactose, each at 10 µg/dL. For experiments involving D-amino acids (alanine or glycine), the BHI was supplemented with either amino acid (500 mM) The plates were incubated for 24 h (37°C, 150 rpm). Triplicate wells of beads were collected following 2, 4, 6, 8, 12, 12, and 24 h of incubation. Following this incubation, the samples were washed in distilled water and placed into a tube containing 2 mL of dissolving solution (5.3 g NaCO₃ and 5.2 g citric acid in 100 ml). The beads were homogenized, and samples were diluted and the bacterial numbers determined by plating on BHI agar and determining the numbers of viable bacteria recovered. These tests were made according to the criteria mentioned by the Clinical Laboratory Standard Institute (CLSI). They were checked with the determination of the stability of growth in terms of CFU with the presence and absence of ciprofloxacin.

**Effects of ciprofloxacin, monosaccharides, and D-amino acids**

In some experiments the antimicrobial action of ciprofloxacin (MHOX; Prepared Media Laboratories, Tualatin, Oreg) was investigated against two *P.
**P. aeruginosa** isolates. One isolate (#1) was challenged with 900 µg/mL of the antibiotic; the second isolate (#2) was challenged with 800 µg/mL.

Similarly, the effects of adding remint. Glucose, galactose, and fructose [BDH, England] were investigated. 12

The effects of D-amino acids were also explored using 500 mM D-alanine and D-glycine, alone or in combination, on sessile and planktonic cells.

**Dual-species biofilms**

*P. aeruginosa* was cultured with isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Overnight cultures of the target bacterial species were re-suspended in BHI broth (1 mL) to a density of approximately 10⁸ CFU/mL; 300 µl of two cultures 1 total bacterial cell volume from both bacteria in 1:1 ratio, then it was vortexing. *P. aeruginosa* was vortexing with *S. aureus*, *K. pneumonia* and *E. coli* (independently). Using 48 wells plate, 600 µl BHI broth containing 1/10 000 from co-culture was pipetting 600 µl into each well. Alginate beads were inserting to the wells after washing them in SDW using flamed wire and pliers. The plate was incubating overnight at 37°C and 150 rpm. Triplicate beads were used for each mixed culture. Media and non-adherent cells were discarded after incubation. They were putting by the experiment in a new medium with ciprofloxacin and without ciprofloxacin. Then Incubate overnight at 37°C, 150 rpm. The beads were collecting at 2, 4, 6, 8, 12, and 24 h; and washed in 600 µl dH₂O in a 48 well plate before dissolving in a 15 ml falcon tube in two ml dissolving solution, the beads were smashing with a sterile loop stick. In order to differentiate between bacteria in the Miles Misra technique, biofilm production was estimated for each bacterium (alone and together), using selective media and antibiotics.

In dual-species biofilms between the studies isolate no. 2 and *S. aureus*, *P. aeruginosa* was inhibited by adding four µg/ml of amikacin to the culture media. Also, the isolate no. 2 was inhibited by exposing to 4 µg/ml of ceftazidime when it is co-cultured with *K. pneumonia*. Further, in dual culture with *E. coli*, the isolate no. 2 was inhibited by two µg/ml of gentamicin. The calculation of CFU for all isolates which were co-cultured with isolate no. 1 was done according to the following equation: CFU of dual-species biofilms culture minus CFU of isolate no. 1. All other above bacteria were inhibiting by cetrimide agar contains naldixic acid. 13

Data analyses were performed using the SPSS-23 (SPSS, Chicago, IL, USA) statistical package. Data are presented as simple measures of frequency, percentages, means, standard deviations, and ranges (minimum-maximum values). For quantitative data, differences between means were examined using t-tests for independent means or paired t-tests for differences between paired observations. An analysis of variance (ANOVA) was used to examine differences between more than two independent means. For qualitative data, the chi-square test was used to examine differences between percentages. P-values ≤ 0.05 were considered significant.

**Results**

The increase in the number of *P. aeruginosa* cells (isolates 1 and 2) attached to the alginate beads, under control conditions (BHI only, no additional antimicrobial or other additive) is shown in Figure 1. There was complete inhibition of growth when cultivating sessile cells of *P. aeruginosa* overnight on alginate beads when the BHI broth either 800 or 900 µg/mL ciprofloxacin for isolates no. 2 and 1, respectively (Figure 1). However, when the sessile cells are actively growing on the alginate beads and producing the biofilms, there is low-level resistance, and after four hours incubation, the ciprofloxacin concentration has a negligible effect on the sessile cells. The development of survival strategy against the selected ciprofloxacin concentration with both isolates only occurs between 4 and 8 hours growth. However, the survival of sessile cells decreased sharply and by 12 hours to isolate no. 2 and 24 hours to isolate no. 1 (figure 1).
The number of cells that adhered to the alginate beads was dependent on both concentrations of the supplements added and the bacterial isolate. The sessile *P. aeruginosa* cells exhibited copious growth when glucose or galactose was added to the media, although much more growth was discernible in the presence of glucose (Figures 2). Glucose supplementation resulted in more adherent cells than either fructose or galactose, and both isolates responded similarly to the added sugar (Figures 2). Both study isolates were yielded, enhancing biofilm production with the addition of glucose (P<0.05). Significant differences were detected in the biofilm production for both isolates with the presence of galactose (P<0.05) with one log increase, compared to sessile cells without any addition. No significant differences observed in the biofilm production with fructose supplementation compared to sessile cells without any addition (Figure 2).

In planktonic stage, the results obtained by adding monosaccharides to the culture medium indicated that the number of free swimming pseudomonal cells increased in two log with the addition of glucose, but with galactose and fructose the increase was negligible. Further, when ciprofloxacin was added the values of MICs were decreased (resistance decrease) with the addition of glucose, while the effect with the presence of galactose and fructose is negligible.
Figure 2. Development of biofilms on alginate beads of two isolates no. 2 and 1, with the addition of different monosaccharides to the culture media using logarithmic panel. Cultures were set up from overnight cultures as described in Materials and Methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars. Dashed line indicates the detection inhibit sessile cells A: glucose supplement, and glucose with ciprofloxacin supplements, B: fructose supplement, and fructose with ciprofloxacin supplements, C: galactose supplement, and galactose with ciprofloxacin supplements.
The combined form of fructose with each glucose and galactose were supplementing to the culture media to determine the biofilm for producer isolates of *P. aeruginosa* during 24 h and compared with sessile cells of *P. aeruginosa* without any addition. The results revealed no significant differences for both isolates. The combined form of glucose and galactose was adding to media, which contains alginate beads to estimate the effect of combined action of glucose and galactose on the activity of sessile cells. They were showing significant differences (P<0.05) with one log increase in comparison with sessile cells of bacteria without addition (figure 3).

**Figure 3.** Development of biofilms on alginate beads of two isolates no. 2 and 1, with the addition of a different combined form of monosaccharides to the culture media. Cultures were set up from overnight cultures as described in materials and methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars. The dashed line indicates the detection inhibit sessile cells. A: glucose with fructose supplements, and glucose, fructose and ciprofloxacin supplements, B: glucose with galactose supplements, and glucose, galactose and ciprofloxacin supplements, C: galactose with fructose supplements, and galactose, fructose and ciprofloxacin supplements.
Ciprofloxacin was added to the media, which contains glucose, fructose, and galactose, the combined form of glucose with each fructose and galactose, and combined form of fructose with galactose. For the study, isolates no. 1 and 2, there were significant differences (P˂0.05) when ciprofloxacin added to culture media containing glucose. The effect of ciprofloxacin on the sessile cells in the presence of glucose is limited and it will become more effective and reduced sharply when the glucose is absent (in absence of glucose) (figure 1); ciprofloxacin did not eradicate the sessile cells with glucose presence. Furthermore, galactose enhanced survival of sessile cells with ciprofloxacin presence, but less than that occurred with glucose addition, as well as with the combined form of glucose and galactose addition with one log increase (P<0.05) compared with BIC of ciprofloxacin in the same time. Other additions when compared with BIC of ciprofloxacin statistically showing no significant differences when added ciprofloxacin to the culture media that containing fructose or combined form of fructose with each glucose and galactose during 24 h, which means that there was no noticeable increase or reduce in P. aeruginosa sessile cells (figure 4).

Figure 4. Development of biofilms on alginate beads of isolate no.2 without any addition, with adding glucose and with adding glucose with the ciprofloxacin. Cultures were set up from overnight cultures as described in Materials and Methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars.

Our initial experiments showed that D-gly, D-ala, and combined form of this amino acids, decreased biofilms formed by P. aeruginosa. However, the inhibitory action of the combined form of D-gly and D-ala was significantly (P<0.05) higher than D-gly and D-ala alone, while D-ala did not affect on sessile cells of isolate no.1. Hence, these amino acids were employed in experiments on account of its efficacy in combined action with ciprofloxacin. A positive correlation between these amino acids and ciprofloxacin was obtained from CFU count for both isolates. From the correlation lines, the number of adherent cells was calculated in the presence of D-gly, D-ala, and combined form of these D-amino acids supplementary. Rates of biofilm formation were obtained from the slopes of regression lines. Our results showed that a marked decrease in sessile cells when adding ciprofloxacin to the media containing D-amino acids. At 12 h, the BIC was observed with synergistic action between D-gly and ciprofloxacin for both isolates, and it was more potent with isolate no. 2. Also, the synergistic action between ciprofloxacin and combined form of D-gly with D-ala was observed. Synergistic
action also observed between D-ala and ciprofloxacin against isolate no. 2 (figure 5). The planktonic cells affected on amino acids. Planktonic cells showed a marked decrease in number with the addition of combined form of D-gly and D-ala, in comparison with D-gly alone. No observed effect appeared with the addition of the D-ala. Further, when adding the ciprofloxacin, the results showed synergistic reaction with the amino acids.

Figure 5. Development of biofilms on alginate beads for isolate no.2 versus addition some D-amino acids and ciprofloxacin to the media. Cultures were set up from overnight cultures as described in Materials and Methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars. The dashed line indicates the detection inhibit of sessile cells A: Sessile cells level without additions, and sessile cells level with added D-amino acids, B: Sessile cells level without any addition, D-amino acids, and ciprofloxacin addition.
Dual-species biofilms occur via a combination of bacterial auto/co-aggregation and attachment to a substratum. As dual-species biofilms under laboratory conditions, two isolates of *P. aeruginosa* with *S. aureus*, *K. pneumonia*, and *E. coli* were cultured for 24 h. They observed in 2, 4, 6, 8, 12, and 24 h. When estimated, the sessile cells compared to the single-species biofilm of *P. aeruginosa* and *S. aureus*, for the same sessile cells number of time. *P. aeruginosa* was increased of sessile cells in co-culture with *S. aureus*, while gave decreased in number in co-culture with *K. Pneumonia* and *E. coli*. However, changes were calculated relative to single-species biofilms for each bacterium. Dual-species biofilm composition analysis of sessile cells and calculation of competitive relative indicated that *S. aureus* reduced at each time point with less competitive relative indices than *P. aeruginosa*. The relative competitors are derived from sessile cell number and more significant competitive indexes, therefore mean higher bacterial numbers. This suggests that it was advantageous for *P. aeruginosa* to be part of a dual-species biofilm in terms of synergistic growth with *S. aureus*. The reduction of sessile cells of *S. aureus* after co-culture was significant differences (P<0.05) with one log increase (figure 6).

![Figure 6](image)

**Figure 6.** Development of biofilms on alginate beads for two isolates no.1 and 2 when co-cultured with *S. aureus*. Cultures were set up from overnight cultures as described in Materials and Methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars. A: isolate no. 1, B: isolate no.2.
Conversely, sessile cells of *P. aeruginosa* were observed when co-incubated with *K. pneumonia* and *E. coli*. Sessile cells of both isolates no. 1 and 2, which were one log reduce (P<0.05) compared to the sessile cells alone, while sessile cells of *K. pneumonia* with both isolates of *P. aeruginosa* no. 1 and 2 were one log increase (P<0.05) compared to sessile cells of *K. pneumonia* alone. Statistically, *E. coli* gave no significant differences compared to *E. coli* alone (figure 7).

Figure 7. Development of biofilms on alginate beads for two isolates no.1 and 2 when co-cultured with *K. pneumonia*. Cultures were set up from overnight cultures as described in Materials and Methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars. , A: isolate no. 1, B: isolate no.2. A: isolate no. 2, B: isolate no. 1.
P. aeruginosa was cultured in the presence of S. aureus, K. pneumonia, and E. coli when grown on alginate beads. We were hypothesizing that exposure of P. aeruginosa to S. aureus, K. pneumonia, and E. coli might alter the BIC of P. aeruginosa to ciprofloxacin. To test this hypothesis, it was selecting a methicillin-resistance S. aureus, K. pneumonia, and E. coli. BIC was determined in 24 h for both isolates of P. aeruginosa with S. aureus. It was 900 µg/ml ciprofloxacin for isolates no. 1, and 800 µg/ml ciprofloxacin for isolate no. 2. After dual-species biofilms culture, they were become 800 µg/ml to isolate no. 1, whilst isolate no. 2 still 800 µg/ml. S. aureus with isolate no.1 before and after dual-species biofilms. BIC was still 1000 µg/ml. S. aureus with isolate no.2 before dual-species biofilms, BIC was 1000 µg/ml, while after dual-species biofilms culture, BIC has become 800 µg/ml (figure 6).

Dual species biofilms with ciprofloxacin addition, P. aeruginosa with K. pneumonia when had co-cultured on alginate beads, BIC was 900 µg/ml ciprofloxacin for isolate no. 1, while BIC of P. aeruginosa isolate no. 2 was 800 µg/ml. After co-incubation with K. pneumonia on alginate beads, BIC becomes 100 µg/ml at four hours of isolate no. 2. Isolate no. 1 becomes 200 µg/ml at the same time. K. pneumonia before co-cultured, BIC was 100 µg/ml, while after co-cultured, it becomes 700 µg/ml with isolate no. 2. With isolate no. 1 BIC of K. pneumonia becomes 300 µg/ml (figure 7).

P. aeruginosa biofilm cell viability increased in 24 h when these microbes were co-culture with E. coli on alginate beads. With ciprofloxacin addition, BIC of P. aeruginosa isolates no. 2 becomes 500 µg/ml. Before co-cultured, BIC was 800 µg/ml. Isolate no. 1, BIC was 900 µg/ml, after co-cultured with E. coli BIC becomes 700 µg/ml. BIC of E. coli was describing before and after co-culture. It was 100 µg/ml ciprofloxacin before dual-species biofilms culture. E. coli when co-cultured with isolate no. 1; BIC becomes 200 µg/ml, while when co-cultured with isolate no. 2; BIC becomes 600 µg/ml (figure 8).

![Figure 8. Development of biofilms on alginate beads for two isolates no.1 and 2 when co-cultured with E. coli. Cultures were set up from overnight cultures as described in Materials and Methods, with triplicate beads or wells and sampled at the indicated time points. Beads were washed, dissolved, serially diluted and plated for CFU/ml counts. Mean CFU/ml counts are plotted against time of growth with error bars. , A: isolate no. 1, B: isolate no.2.](image-url)
Discussion

The impact of sugars and other factors on biofilm formation can vary depending on the type of bacteria present. Up to our simple knowledge, this study has been done for the 1st time in our country. Our research suggested that the fructose and combined form of fructose with each glucose and galactose were at least stop the effect of these two types of sugars when this combined form tested with ciprofloxacin against pseudomonal biofilm. Further, this research suggested that the glucose and galactose affect markedly on sessile cells of P. aeruginosa, and it is in agreement with those observed by Rasamiravaka. who concluded that the glucose could influence growth of biofilm. Geerlings and associates, going to that the urine samples with glucose concentrations between 100 and 1000 mg/dL, were responsible for enhancing bacterial growth, while Khangholi and Jamalli, revealed that the bacterial biofilm was not influenced with the presence of sugar. The interpretation of the above statements is may be due to that the P. aeruginosa had generated at least three polysaccharides (alginate, pel, and psl), which are essential for biofilm structure stability and the qualitative composition in the biofilm matrix of their polysaccharides, primarily alginate, Psl, and Pel, varies in the P. aeruginosa. The Pel polysaccharide is predominantly a glucose-rich matrix, whereas the Psl consists of D-mannose, L-rhamnose, glucose, and repeated pentasaccharides. In the early stages of biofilm formation, Pel and Psl may serve as a primary structure ground for biofilm development. This may also be interpreted as what’s has happened in diabetic patients in vivo who were more likely to get an infection from the urinary tract due to elevated levels of blood sugar, the high concentration of sugar provides to pathogenic bacteria a suitable growth environment.

Our findings showed that the galactose enhancing biofilm formation after 24 hours. This result is in line with Khangholi et al., who showed that galactose enhances biofilm formation. This may be due to the PSL production provides a benefit at the group level to cells growing in biofilms, and Psl is a galactose-rich exopolysaccharide. The study results revealed that the glucose and galactose and combined form with each other, influencing survival strategy against ciprofloxacin. This result is in line with Allison et al., who concluded that after the addition of particular carbon metabolites to persister cells, the cells return into a state in contradiction of which antibiotics. In contrast, Flume et al., reported that adjunctive sugar therapy might be particularly useful in treating P. aeruginosa pulmonary infections with increased intrinsic resistance to antibiotic and multidrug-resistant isolates. This interpretation may be due to that the sugars in the early stage can promote the interaction of a wide range of bacterial adhesions to form bacterial clusters, such as type IV pilis. The sugars can help to bind or absorb antibiotics. Eventually, in clusters, sugar can alt the metabolic status of bacteria without raising the proliferation of bacteria, making them more antibiotic susceptibility.

The free swimming pseudomonal cells increased with the addition of glucose. Further, when ciprofloxacin was added the values of MICs were decreased with the addition of glucose. Our results were in line with Paranjape and Shashidhar, who concluded that the glucose increases the respiration which in turn increases the metabolism and cell division rate. Furthermore, the addition of glucose could increase the susceptibility of persister cells to ciprofloxacin only. In general, the bacterial susceptibility can be increased by combining the antibiotics with glucose.

The planktonic cells affected on amino acids. Planktonic cells showed a marked decrease in number with the addition of combined form of D-gly and D-ala, in comparison with D-gly alone. Our initial experiments showed that D-gly, D-ala, and combined form of these amino acids, reduced biofilms formed by P. aeruginosa, relative to control. The combined form of D-gly and D-ala was more potent against biofilm production than those of control. These results are in agreement with Hammes et al., who suggested that the amount of incorporated D-gly or D-ala can decrease sessile cells of bacteria. This interpreted may be due to D-gly, which is integrated into peptidoglycan. D-gly could replace L-alanine in position one and D-ala residues in positions 4 and 5 of the peptide subunit. The Replacing of L-ala in position one with UDP-MurNAc: l-Ala ligases, which suggest a ligase inhibition of peptides with uridine diphosphate-muramic acid accumulation. Modified peptidoglycan precursors are indicated to be produced by weak substrates of some of the enzymes in peptidoglycan synthesis, D-gly effect on sessile cells CFU/ml. Their investigations had shown
that D-gly interferes with several steps of peptidoglycan synthesis. Kao et al., 24 reported that the D-ala did not deter the development of \( P. \) \( aeruginosa \) biofilm, but delaying in biofilm formation.

In order to gain a better understanding of the interactions between different microbial species in this complex environment, the analysis of these various communities is crucial. Recognizing these relationships will lead to the detection of unique goals and therapeutics that were likely to be missed otherwise. \( P. \) \( aeruginosa \) biofilm monospecies inoculated with \( S. \) \( aureus \), \( K. \) \( Pneumoniae \), and \( E. \) \( coli \) as a secondary colonizer showed significant increases and decreases in the number of sessile cells in contrast with single species \( P. \) \( aeruginosa \) biofilm. We hypothesized that the dual culture between \( P. \) \( aeruginosa \) and \( S. \) \( aureus \) exhibits an increase of sessile cells of \( P. \) \( aeruginosa \). Alves et al., 25 showed that the biomass rises detected between \( P. \) \( aeruginosa \) monospecies and dual-species biofilm relative to single-species \( P. \) \( aeruginosa \) biofilms at the same period. Our findings showed a huge, statistically significant difference (\( P \leq 0.05 \)) between \( P. \) \( aeruginosa \) monospecies and dual-species \( S. \) \( aureus \) Biofilm. \( S. \) \( aureus \) was using in biofilm growth tests as a promoter colonizer and improved \( P. \) \( aeruginosa \) attachment. \( S. \) \( aureus \) contributes to the \( P. \) \( aeruginosa \) biofilms formed for 24 h has a secondary colonizer and results in a decrease in the total biofilm biomass of \( S. \) \( aureus \); this effect is not reciprocal. Somehow disrupts by \( S. \) \( aureus \) for \( P. \) \( aeruginosa \) biofilm, perhaps by dispersion or direct bactericidal action. Also, secreted components extracted from \( S. \) \( aureus \) biofilm repeated this effect, which indicates that the dispersal of biofilm is dependent on a secreted element. \( S. \) \( aureus \) is known to create nucleases that prevent or interfere with the production of biofilm in several bacteria pathogens, including \( P. \) \( aeruginosa \). The results of this study were showing clear interactions between \( S. \) \( aureus \) and \( P. \) \( aeruginosa \) that are both competitive and reciprocal in pathogenicity and colonization for each organism.

The interpretation of bacterial-bacterial interactions may be due to an association between the SpA factor and \( P. \) \( aeruginosa \) Psl, the \( S. \) \( aureus \) element (\( S. \) \( aureus \)-specific staphylococcal protein A) SpA, had shown to inhibit the formation of biofilm as measured. Their inability to add SpA to type 4 pili in the case of \( P. \) \( aeruginosa \) was attributed when Psl was not present, and therefore their attachment was reduced. 26 Our finding revealed that the co-incubation between \( P. \) \( aeruginosa \) and \( S. \) \( aureus \) did not change survival against ciprofloxacin, while Orazi and O’Toole, 27 had shown that interspecies bacterial interactions alter antibiotic tolerance in unpredictable ways. Specifically, they found that \( P. \) \( aeruginosa \) protects biofilm and planktonic populations of \( S. \) \( aureus \) from an antibiotic. This interpretation may be due to the high-affinity quinone oxidation of \( P. \) \( aeruginosa \) inhibitor and siderophores, which is defending \( S. \) \( aureus \) biofilms from antibiotic therapy.

Natural biofilms had often were found within both the ecosystem and the host as multi-species populations that differ significantly from monospecies biofilm systems in their composition, structure, and survival strategies against antimicrobial resistance. \( K. \) \( pneumoniae \) and \( P. \) \( aeruginosa \) were two bacteria that were often found together within biofilm-mediated chronic wound infections. The two species that are active in biofilms that are often found together, \( P. \) \( aeruginosa \) and the \( K. \) \( pneumoniae \). 28 In our results, \( P. \) \( aeruginosa \) showed one log reduction of sessile cells when co-cultured with \( K. \) \( pneumoniae \), while Goncalves et al., 29 showed that the anti-biofilm activity of \( K. \) \( pneumoniae \) supernatant against pseudomonal biofilm was indicated no significant effect on biofilm production. The interpretation may be due to The capsular polysaccharide of \( K. \) \( pneumoniae \) is implied in surface adhesion, spacing and order of bacteria in the initial step of biofilm formation, and is required for late biofilm maturation step. Though the initial adhesion of bacteria on the surface constitutes a crucial step in the formation of biofilm, the spread of bacteria on the surface is also another essential factor in the formation of a biofilm, especially in a multispecies highly competitive environment. Some Gram-negative rods such as the highly motile \( P. \) \( aeruginosa \) produce exopolysaccharides that promote their own surface movement during the early stages of biofilm formation. \( K. \) \( pneumoniae \) is a nonmotile Gram-negative rod and may have developed different social strategies leading to surface exclusion of competitors by large capsular polysaccharide production and therefore allowing successful surface colonization. 30

When \( P. \) \( aeruginosa \) co-cultured with \( E. \) \( coli \) as it was occurred in our research, in addition to \( K. \) \( pneumoniae \), \( P. \)
aeruginosa sessile cells were reduced in number. This result is compatible with those observed by Culotti and Packman, who highlighted the relationship between P. aeruginosa and E. coli. P. aeruginosa introduction triggered a growth response that allowed E. coli to develop biofilm. E. coli was continuously deposited in pre-established P. aeruginosa biofilms and also in nutrient agar reduced colonized the interiors of P. aeruginosa clusters. P. aeruginosa grew significantly lower under the same nutritional and flight conditions in mixed cultivations than in monocultures. In contrast, following the introduction of E. coli, P. aeruginosa biofilm biomass decreased. E. coli’s strong antagonism to P. aeruginosa is suggested by the reduction of P. aeruginosa biomass in dual biofilms. Extracellular indole accumulation had already documented in order to reduce the development of E. coli biofilm by impairing cell motility. Indole, which is an E. coli metabolite formed by tryptophanase from the amino acid breaks down by P aeruginosa, and can probably improve E. coli biofilms through the elimination of the growth inhibition induced by extracellular indole. E. coli strains that were indole-producing and indole-deficient, however, similarly increased in biofilms. The indole-deficient E. coli grew poorly in monoculture, but after the introduction of P. aeruginosa was able to form extensive biofilms. The studies with the indole producing strains E. coli observed similar growth patterns. This suggests that indole can play a significant role in E. coli–P. aeruginosa biofilm’s growth behavior.

Conclusion

The study suggested that the findings concerning the impact of monosaccharide and D-amino acids on P. aeruginosa biofilm. There were significant differences between the numbers of sessile cells before and after adding monosaccharide as well as in amino acids (with and without ciprofloxacin), glucose and galactose, and the combination of each one with ciprofloxacin is the enhancing of P. aeruginosa biofilm and increases survival strategy against ciprofloxacin. In contrast, a noticeable biofilm reduction and marked synergistic action for ciprofloxacin have been determined in the combination of the two D-amino acid, alanine, and glycine in comparison to the effect of each one alone. Furthermore, pseudomonal dual-species biofilms culture with S. aureus gave a raised number of sessile cells with the reduction of BIC. In co-culture with K. pneumonia and E. coli, sessile cells of P. aeruginosa were reduced in number and made Pseudomonal biofilm less resistant to ciprofloxacin.

Ethical Clearance- Taken from Committee, University of Anbar, Ramadi, Iraq (approval number 112, October 29, 2019).

Source of Funding- Self

Conflict of Interest- Nil

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Isolation and Analysis of Nucleotide Sequences of the 16S rRNA Gene of *Pseudomonas aeruginosa* Isolated from Clinical Samples

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Abstract

*Pseudomonas aeruginosa* consider one of the opportunistic pathogens is able to infection almost all body tissue as a result of having it a variety of virulence factors that contribute greatly to pathogenic events in the host this bacterium has been responsible for 30% of pneumonia, 19% of urinary tract infections and 10% of bloodstream infections. And leads to nosocomial pathogens causing infections that usually develop late during hospital stay. Consequently the aim of this research was to classify and characterize *P. aeruginosa* isolated from various clinical samples from Iraqi patients by sequencing the 16S rRNA gene.

**Keywords:** Health; Clinical samples; RNA genes; toxicity; *Pseudomonas aeruginosa*

Introduction

*Pseudomonas aeruginosa* is a Gram-negative bacteria belong to the family Pseudomonaceae, motile with a polar flagellum, non-spore forming, obligate aerobes that grows in a wide range of temperatures (10-44 °C) and the optimum thermal temperature is 35°C, widespread in nature, this bacterium causing acute and chronic infections in patients who are in hospital, especially in patients with burns, its grows well when cultured on simple media, It has the ability to produce two kinds of pigment green-blue (pyocyanin) and yellowish green pigment (pyoverdin) [1-3].

This bacterium is a naturally common among humans [4]. It has a relatively large genome, which is likely to promote survival in various environments, with a variety of gene-regulatory activities to facilitate adaptation to new environmental conditions [5]. Therefore, despite the development of new and improved antibiotics is still one of the key causes of death in critically impaired patients with immune systems [6]. 16S rRNA sequencing has been instrumental in the successful detection of bacterial isolates and in the discovery of new isolates in diagnostic laboratories. this gene is especially important for bacterial detection with unique phenotypic profiles, rare bacteria, slow growing bacteria, uncultivable bacteria and culture-negative infections, it isn’t only did it give information into the etiology of an infectious disease, it also allows physicians to pick antibiotics and assess the length of care and infection prevention procedures. [7] 16S rRNA sequencing has provided important and valuable knowledge worldwide.

Materials and Methods

One hundred of specimens were collected (46 males and 54 females) from five hospitals in Baghdad, including: ghazi Al-hareri Hospital, Wound and Burn Hospital, Baghdad Medical City Hospital, Child Teaching Hospital, and Ibn Al-baladi Hospital, from September to December 2019. Collected from patients with various infections such as UTIs, burns, wounds, pus swab from ear infection and sputum. All specimens were cultured on MacConkey agar (selective and differential medium). Then used the biochemical tests for identify and recognize Gram negative bacteria, Also GN24 Kit had used to detect Pseudomonas species.

DNA Extraction

For 16S rRNA sequencing analysis, genomic
DNA has been extracted for (38) isolates belong to *P. aeruginosa* using ABIOpure Extraction Kit (USA). Then use Quantus Florometer to measure the concentration of collected DNA to measure the sampling goodness for downstream applications. All bacterial DNA has been used as a template for conventional PCR amplification bacterial 16S rRNA gene were amplified by tow universal primers 27F (5’ AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’TACGGTTACCTTGTTACGACTT-3’) for forward and reverse primers (Macrogen, South Korea). Thermal cycling conditions were as follows: 5 min of 95 °C for initial denaturation (30 cycles of denaturation) annealing (60°C for 30 sec), extension (72°C for 30 sec); Final extension at 72 for 7 min (1 cycle). PCR was carried out on a (Bio-Rad, USA) Automatic Cycler Thermal. Duplicate PCR was performed for replication of each sample [9]. After amplification with PCR, the agarose gel electrophoresis was followed to validate the amplification existence.

**Bacterial 16S rRNA sequences analysis**

16S rRNA gene were amplified by using universal primers and the PCR product undergo for sequence analysis in Macrogen Lab. (south Korea) the sequences were identified by alignment and comparing them with sequences deposited in genbank through the NCBI site.

**Phylogenetic analysis**

The sequences of 16s rRNA compared with those in deposited in genbank database (https://www.ncbi.nlm.nih.gov/blast) to find related species and creation Phylogenetic trees using geneious software version 11.1 [10]

**Results and Discussion**

One hundred (100) clinical samples were collected from different infections include (wounds, burns, urine, ear infections and sputum), 38 (38%) isolates belonging to *P. aeruginosa*, whereas the remaining 62 (62%) isolates belonged to other bacteria species as seen in the table (1).

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>No. of isolate</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>39</td>
<td>39%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>38</td>
<td>38%</td>
</tr>
<tr>
<td>Klebsella spp.</td>
<td>12</td>
<td>12%</td>
</tr>
<tr>
<td><em>Proteus spp.</em></td>
<td>7</td>
<td>7%</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>4</td>
<td>4%</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>100</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Thirty eight (38) isolates from the total clinical isolate were classified by traditional methods (characteristics, morphology) as *P. aeruginosa*. In addition to use Identification system for Gram negative bacteria (GN24 Kit) the results are shown in figure (1,2):
Kit GN 24 consists of 24 wells Microtitration plate strip in the classic 96 well format containing dehydrated substrates. Reconstitution of substrates is performed by bacterial suspension inoculation. During incubation, color changes occur in wells due to microorganisms ‘ metabolic activity. Test results may be measured either by automated readers or visually depending on the color scheme, or by the color definition displayed in the flyer. The results of recognition can be collected from the evaluation table or by using evaluation tools located at (www.diagnostics.sk/idmicro).

Conventional polymerase chain reaction PCR

The results of Conventional polymerase chain reaction followed electrophoresis to detect of *P. aeruginosa* using 16S rRNA that previously identification by GN24 Kit showed all isolates were *P. aeruginosa* and gave a good confirmative identification as shown in the figure (3). That gives the band size (1500 bp) which was the product size of primers used for identification.

![Image of 16S rRNA gel electrophoresis](image3.jpg)

Figure (3) Results of the amplification of 16S rRNA gene of *Pseudomonas aeruginosa* samples fractionated on 1% agaros gel electrophoresis stained by Eth.Br. Lane1:100bp DNA marker.
16S rRNA gene sequences contain hyper variable regions with high conservation which have potential to identify species-specific signature sequences that are helpful to the classification of bacteria \[11,12\]. Analysis sequencing of this gene consider as an important method to assess the phylogenetic relationship between strains. The properties of this molecular target make it important and beneficial for the detection and diagnosis of bacteria in the clinical laboratory.

**Bioinformatics analysis**

The 16S rRNA nucleotide sequences scanned for similarity sequences using online BLASTn. And use geneious software version 11.1. To create phylogenetic tree for 12 isolates selected randomly, the result tend to have a strong identical between them and identical with those strains deposited in genbank under accession numbers China KF894970; Iran MG016493; China MK825339; Bangladesh MN256396; India K675975; India KX268504; China KT759043. The phylogenetic tree for this gene appears all of the isolates have a common ancestor. (Figure 4)

![Figure 4: Construction of phylogeny tree for twelve isolates of *P. aeruginosa* using geneious software, appear all of the isolates has a common ancestor.](image)

16S rRNA sequence has long been used as a “gold standard” taxonomy in the determination of the phylogeny of bacterial species. In the last decade, the *Pseudomonas* classification has gained further attention and has been reclassified by Brosch *et al* 1996, Kersters *et al* 1996, Palleroni 1992. \[13-15\]. Identification of *Pseudomonas* is causing a lot of difficulties \[16, 17\]. Morphologically related organisms have a like biochemical properties. Sequence of highly conserved gene area 16S rRNA data allows us to predict accurate taxonomy. Our present research was performed on the 16S rRNA sequence based on PCR amplification for recognition and genetic level identification of *Pseudomonas aeruginosa*.

**Conclusion**

In conclusion, PCR assay based on 16S rRNA sequencing is highly precise, responsive and useful in the detection of bacteria. And use for recognition of
those closely connected ones genotypical species of Pseudomonas. DNA sequencing of the 16S rRNA gene was used as an important method for studying Bacterial phylogeny and taxonomy relationships between strains.

Acknowledgment

We gratefully thank for University of Babylon, Science College for Women for their kind supporting and agree to performed this work in its Laboratories and hospitals in Baghdad medical city for supporting and supplying the clinical samples for this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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The Effects of Different Intensive Physical Performances by athletes on Selected Hematological Parameters

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Abstract
The aim of this review study is to compare the effects of different intensive physical performances and competitions from several studies include (football, basketball, 24h marathon race and cycling) games on hematological parameters of males players. And to find the one with strongest effect on those parameters among the performances. The Hematological parameter include white blood cell count (WBC) , red blood cell count (RBC) count , hemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV) , mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT). Iron and Feritten level also estimated in one study. When comparing the blood values for all the players before and after the competitions period, there were significant differences in prevalence of most of blood parameters in all studies but the most impact was in 24 h marathon performance of 11 male runners who raced for 24 hours around a 400 m oval track, covering at least 100 km of distance. The runners changed course every 4 hours and were allowed to rest and freely consume food and water. The blood samples were obtained from subject in 2 and 9 days prior to competition and after competition. The result was showing that the runners suffering from distinguish blood anemia appear after competition in 2 day. The findings show that the hematological parameters could be affected by endurance of performance depending on competition time period and intensity of physical performance.

Keyword: physical performances, hematological parameters , WBC , RBC, HGB, HCT, MCV, MCHC, PLT, anemia.

Introduction
Physical and physiological changes in virtually all organs and structures within the human body can occur during physical activity. Changes that occur in the organism can be observed by using the right experimental techniques, and what needs to be done in this direction can be determined. It is possible to achieve physiological adaptation to the different athlete training environments in the context of the data decided on the basis of the research (1). In the relatively stable population, hematological parameters are determined by many factors. Those factors include education, age , sex, ethnicity, diet, and altitude (2). Each or all of these factors may affect hematocrit (Hct), hemoglobin (Hb), and red blood cell (RBC) counts positively or negatively. In particular, the resistance training often decreases all three (3).

To our knowledge, Longitudinal studies are lacking in highly trained adolescent athletes evaluating the intensity of hematological, hormonal and enzymatic behavior over an annual training period. Training may have positive or negative effects on development, metabolites, enzymes and haematological variables depending on the training load, specificity of the training, age and initial training level (4). Hematological and biochemical parameters can differ by type, intensity, exercise duration, feeding status, and supplementation (5). The hemoglobin and hematocrit values of athletes undergoing intense exercise program
decrease characteristically and this status is considered to be anemia among sportsmen (6). The energy consumption and metabolism arising from long term workouts and competitions expand in the body. Increased metabolites cause a gradual decline in muscle and nervous system function that leads to exhausting chemical, physiological, psychological and environmental factors in this exhaustion. The produced blood metabolites are lactic and pyruvic acid arising from the digestion of carbohydrates, urea, uric acid, phosphates, creatinine and creatinine as a result of the metabolism of proteins, acetone and ketone bodies as a consequence of fat metabolism. Besides, the chemical causes of fatigue are decrease in blood sugar, hypoglycemia and decrease in the amount of oxygen (7).

Although vigorous exercise in the athletes is causing exhaustion, the organism recovers with sufficient rest. Afterwards the individual’s physical ability increases. Unless the rest time after intense workouts is inadequate for rehabilitation, due to the prolongation of fatigue life, the physical capacity is degraded (8). The hematocrit, hemoglobin, Fe, ferritin, calcium, magnesium, urea, uric acid, creatine, creatine kinase, CPK (creatine phosphokinase), and total protein should be measured to assess the overtraining, which is a loss of efficiency due to physical and mental exhaustion (9).

The aim of this study is to compare the changes that may occur in the blood values before and after intensive exercise in different physical performances in different player groups in several studies and to find which performance has more effective influence on hematology parameters.

Methodology

The methodology of this study will review the method and results of four different studies with varies physical performances include football, basketball, 24 h marathon and cycling.

Study by Ayhan et al., (2017) on 28 soccers athletes two-stage tests were performed pre-competition and post-competition test (90 minutes official competition) the pre-competition test was on rest day no competition and training. To assess the subjects metabolic, biochemical, and haematological values. In field conditions (natural grass surface football field) post-competition experiments were carried out. The intergroup pre- and post-test variables of the teams were determined using independent t test. Study of Pearson correlation has been used for the relationship between variables within each team. If P < 0.05, a statistically relevant result will be acknowledged (10). Study by Gencer et al., (2018) was performed on 10 volunteer basketball players with an average age of 22.80 ±3.20 years and average height of 185.83 ±7.57 cm. Teams without health issues were university graduates. Before and after competitions (24 hours) with potassium-edged tubes for hemogram 5 ml of blood samples were taken by venous pathway. The model CELL-DYN-3500 R automated blood counting system was used for laboratory research. The SPSS package software was applied to descriptive statistics data and the two sample experiments carried out by Wilcoxon were used to compare values before and after competition (11).

Study by Wu et al., (2004) In this research 11 runners had previously given their informed consent. The runners raced for 24 hours around a 400 m oval track, covering at least 100 km of distance. Each 4 hours the runners changed course. 24 hours before the run, directly after the race, two days after the race and nine days after the race, blood samples were obtained from the antecubital vein. The blood was tested within 1 h with an autoanalyzer ABBOTT CELL DYN 3 000 (Abbott Diagnostics, Mountain View, CA., USA) and HITACHI 7150 (Hitachi High Techotologies, Tokyo, Japan).

The statistical significance of paired variations in mean and standard deviations of the associated hematological and biochemical changes between pre-race, immediate post-race, two days post-race and nine days post-race values were determined using ANOVA analyzes of one type. The significance level was set to P<0.05 (12). A total of 272 cyclists male subjects were contribute in the study by Schumacher et a., (2002). Under standardized conditions, blood sampling took place in a supine position on an empty stomach before race and after 9 days of race in the morning. Blood was drawn into a 4-mL K-EDTA coated vacutainer device from a cubital vein. The following variables were evaluated within 3 h of the prelevation in the EDTA samples by an automated cell counter (Serono-Baker Diagnostics, Allentown, PA; Model 9000 Diff): Hb, Hct (percent), and red blood cell count (106·mm 3) (13).
Results and Discussion

Study by Ayhan et al., (2017) result were Red Blood Cell RBC, Hemoglobin HGB, Hematocrit HCT, Mean Erythrocyte Volume MCV, Mean Hemoglobin MCH, Erythrocyte Hemoglobin Concentration MCHC, Trbomocyte PLT and statistically significant values (p>0.05). (Tab. 1). White Blood Cell WBC values were found to be statistically significant (p < 0.05) in athletes category after performance compared to before performance (10). Bezci et al found that athletes had a significant increase in WBC rates in their country, European and Turkey youth categories research on athletes (14).

There is a significant increase in the prevalence of, MCH, MCHC values before and after the competition, as shown in the Gencer et al (2018) Table 1 report. The HCT and MPV values decreased significantly (P<0.05). The findings show that intensive competitions may have affected the physiological characteristics of the basketball players. There are reports

<table>
<thead>
<tr>
<th>Study and Performance</th>
<th>Hematological P</th>
<th>Before performance</th>
<th>After performance</th>
<th>P valu</th>
</tr>
</thead>
<tbody>
<tr>
<td>football players</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n= 28 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (K/ul)</td>
<td>5.40±1.63</td>
<td>8.62±2.96</td>
<td>0.005**</td>
<td></td>
</tr>
<tr>
<td>RBC (K/ul)</td>
<td>6.40±1.63</td>
<td>5.64±0.73</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>15.52±1.36</td>
<td>14.04±0.97</td>
<td>0.778</td>
<td></td>
</tr>
<tr>
<td>HCT (%)</td>
<td>44.03±2.45</td>
<td>43.60±1.65</td>
<td>0.300</td>
<td></td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>82.45±9.83</td>
<td>83.28±9.18</td>
<td>0.331</td>
<td></td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27.29±4.18</td>
<td>27.4±4.02</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>32.97±1.97</td>
<td>33.12±1.47</td>
<td>0.925</td>
<td></td>
</tr>
<tr>
<td>PLT (K/uk)</td>
<td>234.92±71.71</td>
<td>286.80±61.98</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>basketball players</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n= 10 (11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (K/ul)</td>
<td>7.04±1.34</td>
<td>7.06±1.67</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>RBC (K/ul)</td>
<td>5.72±0.53</td>
<td>5.59±0.49</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>16.05±,88</td>
<td>15.96±1.15</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>HCT (%)</td>
<td>48.52±2.82</td>
<td>47.72±3.12</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>85.24±6.62</td>
<td>84.88±6.50</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.21±2.32</td>
<td>28.53±2.33</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.06±4.45</td>
<td>33.56±4.45</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>PLT (K/uk)</td>
<td>222.5±51.02</td>
<td>211.5±46.65</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>24 h marathon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>runners n=11 (12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (K/ul)</td>
<td>4.95±1.05ace</td>
<td>6.95±1.45</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>RBC (K/ul)</td>
<td>4.71±0.25</td>
<td>3.42±0.21</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>14.63±0.91ce</td>
<td>12.81±0.69</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>HCT (%)</td>
<td>42.34±2.73ace</td>
<td>37.27±1.84</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>89.91±3.11e</td>
<td>90.15±3.19</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.09±1.23</td>
<td>30.22±1.34</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>PLT (K/uk)</td>
<td>235.45±47.27</td>
<td>209.27±67.23</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Cyclists n= 272 (13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>5.24 ± 0.52</td>
<td>5.15  0.41</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>15.72 ± 1.02</td>
<td>14.66 ± 1</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>HCT (%)</td>
<td>46.6 ± 3.3</td>
<td>46.3 ± 3.1</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>125 ± 50.23</td>
<td>116.2 ± 37</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>125.6 ± 91.5</td>
<td>110.3 ± 131.3</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1. Hematological variables of various sporting categories in different studies before and after performance
in the literature on the acute impact of exercise on blood parameters. Significant increases were found at WBC, values (15). Chronic effects of exercise on blood parameters have been documented in some other research. Hematocritic and hemoglobin levels have been shown to be in activation (16). There are, however, not enough research on the impact of prolonged duration of competition on blood parameters. (17) analyzed the blood parameters of soccer players during the 10-day intense competition period and found a significant impact on the values of RBC (Erythrocyte), PLT (Platelet) and HGB (Hemoglobin) (p<0.05) and statistically insignificant effect on the values of WBC (Leukocyte) (p>0.05). Fewer blood parameters were analyzed in this analysis, and blood samples were taken 2 hours after the conclusion of the vigorous competition. Blood samples were taken the day after the research to rule out the acute impact of vigorous exercise (11). Study by Wu et al (2004) on 24-hour marathon runners shows a substantial decrease in red cell count, Hb and Hct rates by day two after race (Table-1). The mean concentration of cell Hb on day two was slightly lower than before the race. Mean cell Hb and the red cell distribution width remained constant at all times. Red cell count, Hb and Hct, three anemia markers, had been normal prior to the race. Consistent with RBC’s rapid deterioration of endurance athletes, substantial declines were noticed by day two (18). Among days two and nine the three factors remained reduced; so-called sports anemia. The results and observations of vigorous training on athlete safety can be used to support athletes in future competitions. Is caused not only by hemolysis due to mechanical trauma but also by red cell oxidizing injuries (19). Red cells with a mean life of 120 d are renewed at approximately 1 per cent daily under normal conditions. However, as demonstrated in the participants in this study, this turnover rate increases after the endurance training. The increased turnover rate is beneficial for athletes because young red cells can be more effective in carrying oxygen than older cells (19). The mean volume of cells, the mean concentration of cell hemoglobin and the mean concentration of cell hemoglobin all remained normal. Transient sports anemia has been caused by reduced red cell numbers, rather than red cell size or Hb volume (20). The adjustment to the number of platelets was inconsistent with previous studies. At the end of the race the platelet count was higher than on day nine, but stayed within the normal range, and no coagulopathy was observed. The number of white cells increased markedly and decreased thereafter (12).

Study by Schumacher et al (2002) on 272 Cyclists before and after performance showed that a significant decrease in iron levels and a significant decrease in ferritin levels Iron and especially ferritin, the iron storage protein, was shown to be reduced in athletes due to higher iron turnover and increased synthesis of iron-containing proteins combined with altered intestinal absorption and an increase in iron-containing proteins. The results from the study highlights these findings for athletes as there is a substantial decrease in ferritin relative to sedentary citizens. Hemolysis caused by exercise, frequently discussed as a cause for decreased levels of iron and ferritin, or even anemias (13).

Conclusions

This study conclude that the hematological parameters could be affected by endurance of performance depending on competition time period and intensity of physical performance. The most affected players were the 24h marathon runners due to the long period and intensity of exercise. A better understanding of these factors will help event organizers and coaches in the planning of competitions and will make it easier for the sportsmen to prepare for their protection for these intense competitions.

Acknowledgements: The Author would like to thank the Medical Laboratory Technology Department, Collage of Medical Technology, The Islamic University, Najaf, Iraq. for their generous support of this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Role of CoQ10 and IGFBP-1 in Obese Male Patients with Diabetic Mellitus Type II

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Abstract

Study the role of CoQ10 and IGFBP-1 in obese male patients with diabetic mellitus type 2. ELISA method was used to assay Serum CoQ10 and IGFBP-1. Blood was taken with drawn sample from 30 obese normal patients with age range (40-60) years, 30 diabetic patients with age range (40-60) years at duration of disease (1-5) years and 30 normal healthy patients. The mean difference between T2DM according to CoQ10 (12.5±1.1) was decreased than the mean of IFG (21.8±3.2) (P 0.002) and the mean difference between T2DM according to IGFBPs (0.65±0.06) was decreased than the mean of IFG (3.2±0.3) (P 0.000). While no significant difference between mean age of DM2 patients (55.5±1.06), and IFG (55.6±0.9) (p 0.90), no significant difference between mean BMI of DM2 patients (27.7±0.8), and IFG (27.8±0.5) (p 0.94). were significant differences in DM and IFG obese groups (G1 and G2) according to age (51.66 ±2.10, 51.80±1.16) P (0.02), however, there were significant differences between DM and IFG in Normal weight groups (G5 and G6) according to age (59.93±0.94, 51.13±1.80) P (0.00), while no significant differences between DM and IFG in Over weight groups (G3 and G4) according to age (54.93±1.17, 58.00±1.73) P(0.21), there were significant differences between DM2 and IFG in obese groups (G1 and G2) according to BMI (33.70±1.20, 31.1±0.37) P (0.01), no significant difference between overweight (G3 and G4) according to BMI (27.72±0.30, 27.52±0.34) P(0.66), and no significant difference between normal weight (G5 and G6) according to BMI (21.84±0.45, 21.53±0.50) P(0.65). There were significant differences between DM and IFG in obese groups (G1 and G2) according to CoQ10 (7.2±0.4, 4.9±0.4) P (0.002), and IGFBP (0.3±0.02, 1.2±0.19) P (0.005).

Keywords: CoQ10, Obese patients, Diabetic Mellitus Type 2, IFG.

Introduction

Diabetes mellitus (DM) is a chronic disease described by elevated of blood glucose. The raised concentration of blood glucose result from the insufficient production of insulin or an imperviousness to the impacts of insulin, a hormone framed by the pancreas (1). It is becoming one of the main chronic non-contagious diseases threatening the health of human around the world (2). T2DM accounts for between 90% and 95% of diabetes, with highest proportions in low- and middle income countries (2). It is a common and serious global health problem that has evolved in association with rapid cultural, economic and social changes, ageing populations, increasing and unplanned urbanization, dietary changes such as increased consumption of highly processed foods and sugar sweetened beverages, obesity, reduced physical activity, unhealthy lifestyle and behavioural patterns, fetal malnutrition, and increasing fetal exposure to hyperglycemia during pregnancy (3). T2DM is most common in adults, but an increasing number of children and adolescents are also affected (4). Obesity is a disorder characterized by an unequal increase in body weight in relation to height, mainly due to the accumulation of fat. Obesity is considered a pandemic of the present century by the World Health Organization (WHO) and other international organization (5). Obesity is associated with the development of important non-
communicable chronic diseases, namely, hypertension, metabolic syndrome, type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), obstructive sleeping apnea, osteoarthropathies and cancer. Insulin-like growth factor 1 (IGF-1) is a 70-aminoacid polypeptide hormone with endocrine, paracrine, and autocrine effects, which shares structural homology (>60 %) with IGF-2 and proinsulin. It is mainly produced by the liver (accounting for ≈75 % of circulating IGF-1) secondary to growth hormone (GH) and insulin endocrine stimulation in the liver. Conversely, IGF-1 acts to provide an inhibitory feedback signal on GH secretion in the hypothalamus by stimulating somatostatin production in the pituitary. IGF-1 is also produced locally in all bodily tissues. IGF-1 availability is tightly regulated by the so-called insulin-like growth factor binding proteins (IGFBPs), which may act by increasing IGF-1 half-life, from minutes to hours (most commonly by forming a tertiary complex with Acid-Labile Subunit and IGFBP3), however blocking its binding to the insulin-like growth factor 1 receptor (IGF-1R).

Materials and Methods

This study performed during period from September 2019 to December 2019 the subject were selected from Teaching Hospital/Medical City. Questionnaires were filled by participants and to get the agreement to participants in this study to collect the information of control and patients group. Blood samples were collected from control and patients group. The sample was drawn from the vein and stored by using (5mL) disposable syringe, all samples were collected in fasting status. The sample was keep into dispensable tubes containing a gel which facilitate the separation processes of serum and allowed to clot at 37°C approximately at ten-fifteen min and then centrifuged at 2000 Xg for ten-fifteen min then the serum was stored at (-20°C) until analysis (CoQ10 and insulin-like growth Factor Binding Protein -1).

Subjects (patients and control groups):

Subject were enrolled in this study First group: patients 30 normal obese male with age range (40-60) years. Second group: DM type 2 (30) male with age range (40-60) years the duration of disease (1-5) years. Third group: 30 normal healthy male documented by physician or lab investigation matched in their age in both obese group.

Measurement of Human CoQ10.

1. Standard wells, a volume of 50μl of the standard solutions were added to the standard wells. Then a volume of 10μl of the sample was added followed by 40μl of sample diluent was added to the testing sample well, in blank nothing to add. Afterword, a volume of 100μl of HRP-conjugate reagent was added to each well and then covered by used adhesive strip followed by incubation for sixty min at 37°C. Next ,the cover on a plate was removed and starting to wash process, the wash process was repeated for four times using 400μl of Wash Solution each time by an auto washer. After that, a volume of 50μl of chromogen solution (A) and (B) was added to each well and mixed gently and followed by incubation period at 37°C for 15 min. This addition should be protected from light. Then, a volume of 50μl Stop Solution was added to each well. The color in the wells converts from blue color to yellow color. If the color in the wells become green or the color change does not appear uniform, the plate should gently covered to ensure good mixing. Later a microtiter plate reader was used to read the absorption within 15 min at 450 nm. A dose response standard curve was used to evaluate the concentration of CoQ10 in serum.

Measurement of Human Insulin-like growth Factor Binding Protein -1.

Standard wells, a volume of 50μl of the standard solutions were added to the standard wells. Then a volume of 10μl of the sample was added followed by 40μl of sample diluent was added to the testing sample well, in blank nothing to add. Next, a volume of 100μl of HRP-conjugate reagent was added to each well and then covered by used adhesive strip followed by incubation for sixty min at 37°C. The cover on a plate was removed and starting to wash process, the wash process was repeated for four times using 400μl of Wash Solution each time by an auto washer. After that, a volume of 50μl of chromogen solution (A) and (B) was added to each well and mixed gently and followed by incubation period at 37°C for 15 min. This addition should be protected from light. After , a volume of 50μl Stop Solution was added to each well. The color in the wells converts from blue color to yellow color. If the color in the wells become green or the color change does not appear uniform, the plate should gently covered.
to ensure good mixing. Later, a microtiter plate reader was used to read the absorption within 15 min at 450 nm. A dose response standard curve was used to evaluate the concentration of (IGFBP-1) in serum.

Statistical Analysis

The version twenty of SPSS was used to complete Statistical analysis. (Means ± SD) were used to represent the variables. The comparison between patients group and control group was done by use student t-test; with a p-value of ≤ 0.001 was considered a significant. The method that used to find the relationship between two continuous variables was correlation coefficient (r).

Results

The result showed as in Table (1) and the mean difference between T2DM according to CoQ10 (12.5±1.1) was decreased than the mean of IFG (21.8±3.2) (P 0.002) and the mean difference between T2DM according to IGFBPs (0.65±0.06) was decreased than the mean of IFG (3.2±0.3) (P 0.000). While no significant difference between mean age of DM2 patients (55.5±1.06), and IFG (55.6±0.9) (p 0.90), no significant difference between mean BMI of DM2 patients (27.7±0.8), and IFG (27.8±0.5) (p 0.94).

Table (1): The mean difference between DM2 and IFG patients according to parameters in this study

<table>
<thead>
<tr>
<th>parameter</th>
<th>DM2 mean± SE</th>
<th>IFG mean± SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>55.5±1.06</td>
<td>55.6±0.9</td>
<td>0.90</td>
</tr>
<tr>
<td>BMI (Kg/M2)</td>
<td>27.7±0.8</td>
<td>27.8±0.5</td>
<td>0.94</td>
</tr>
<tr>
<td>CoQ10 nmol/L</td>
<td>12.5±1.1</td>
<td>21.8±3.2</td>
<td>0.02</td>
</tr>
<tr>
<td>IGFBP ng/ml</td>
<td>0.65±0.06</td>
<td>3.2±0.3</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Moreover, In Table (2), (3), (4), the mean differences between DM2 and IFG cases in different weight groups (Ob., Ow. and Nw) according to age, BMI, COQ10 and IGFBP were studied, the results showed that, there were significant differences in DM and IFG obese groups (G1 and G2) according to age (51.66 ±2.10, 51.80±1.16) P (0.02), however, there were significant differences between DM and IFG in Normal weight groups (G5 and G6) according to age (59.93±0.94, 51.13±1.80) P (0.00), while no significant differences between DM and IFG in Over weight groups (G3 and G4) according to age (54.93±1.17, 58.00±1.73) p(0.21), there were significant differences between DM2 and IFG in obese groups (G1 and G2) according to BMI (33.70±1.0, 31.11±0.37) P (0.01), ), no significant difference between overweight (G3 and G4) according to BMI (27.72±0.30, 27.52±0.34) P(0.66), and no significant difference between normal weight (G5 and G6) according to BMI (21.84±0.45, 21.53±0.50) P(0.65). There were significant differences between DM and IFG in obese groups (G1 and G2) according to CoQ10 (7.2±0.4, 4.9±0.4) P (0.002), and IGFBP (0.3±0.02, 1.2±0.19) P (0.005). However, there were significant differences between DM and IFG in overweight groups (G3 and G4) according to CoQ10 (23.2±0.4, 64.5±1.6) P (0.00), and IGFBP (1.2±0.03, 4.1±0.2) P (0.00). In addition to that, there were significant differences between DM and IFG in normal weight groups (G5 and G6) according to CoQ10 (7.2±0.4, 12.8±1.6) P (0.003), and IGFBP (0.3±0.01, 6.2±0.3) P (0.00).
Table (2): The mean difference between DM and IFG cases in different weight groups according to age

<table>
<thead>
<tr>
<th>Groups</th>
<th>subgroups</th>
<th>age year mean±SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese DM/G1</td>
<td>51.66±2.10</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>IFG/G2</td>
<td>51.80±1.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over weight DM/G3</td>
<td>54.93±1.17</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>IFG/G4</td>
<td>58.00±1.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight DM/G5</td>
<td>59.93±0.94</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>IFG/G6</td>
<td>51.13±1.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): The mean difference between DM2 & IFG cases in different weight groups according to BMI

<table>
<thead>
<tr>
<th>Groups</th>
<th>subgroups</th>
<th>BMI (Kg/M²) P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese DM/G1</td>
<td>33.70±1.20</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>IFG/G2</td>
<td>31.11±0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over weight DM/G3</td>
<td>27.72±0.30</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>IFG/G4</td>
<td>27.52±0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight DM/G5</td>
<td>21.84±0.45</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>IFG/G6</td>
<td>21.53±0.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4): The mean difference between DM2 & IFG cases in different weight groups according to CoQ10 and IGFBP

<table>
<thead>
<tr>
<th>Groups</th>
<th>subgroups</th>
<th>CoQ10 nmol/L P-value</th>
<th>IGFBP ng/ml P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese DM/G1</td>
<td>7.2±0.4</td>
<td>0.002</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>IFG/G2</td>
<td>4.9±0.4</td>
<td></td>
<td>1.2±0.19</td>
</tr>
<tr>
<td>Over weight DM/G3</td>
<td>23.2±0.4</td>
<td>0.00</td>
<td>1.2±0.03</td>
</tr>
<tr>
<td>IFG/G4</td>
<td>64.5±1.6</td>
<td></td>
<td>4.1±0.2</td>
</tr>
<tr>
<td>Normal weight DM/G5</td>
<td>7.2±0.4</td>
<td>0.003</td>
<td>0.3±0.01</td>
</tr>
<tr>
<td>IFG/G6</td>
<td>12.8±1.6</td>
<td></td>
<td>6.2±0.3</td>
</tr>
</tbody>
</table>
Discussions

These results were agreement with results obtained by Alehagen et al.\(^{(12)}\) who found that, there were significant differences between T2DM and IFG according to CoQ10 (\(P < 0.001\)). Dulskas et al.\(^{(13)}\) found that, there were no significant difference between T2DM and IFG according to age (\(P 0.89\)), BMI (\(P 0.74\)), this result was agreement with results in this study. A study of\(^{(14)}\) found that, there were significant differences IGFBP in patients with T2DM. The clinical parameters were studied by\(^{(15)}\)found that age and BMI, were no significant differences in T2DM patients, so, these results were agreement with results obtained by\(^{(15)}\) who found that, there were significant differences between IFG and CoQ10 (\(P < 0.005\)). Wei et al.,\(^{(16)}\) found that, there were no significant difference between IFG and age, BMI P (0.55), while the results in this study were disagreement with study of\(^{(17)}\) who found that, there was a statistically significant interaction was found between T2DM and BMI (\(p<0.0001\)). The results of\(^{(18)}\) found that, the diabetic patients were not associated with obesity (\(p=0.020\)) and were independent of age. These results were agreement with results obtained by\(^{(19)}\) who found that More than 90% of patients with type 2 diabetes have a BMI ≥25.0 kg/m2. In adult patients with type 2 diabetes, some studies have shown that individuals who lost 9–13 kg had a 25% reduction in all-cause mortality compared to weight-neutral patients. The results were agreement with results obtained by\(^{(18)}\) who found that, increased BMI was associated with increased prevalence of diabetes mellitus (\(p < 0.001\)). In addition to that\(^{(20)}\) found more than 75% of patients had BMI ≥ 25 kg/m2 estimated that prevalence of diabetes mellitus.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References


Histological and Morphological Study of Carcinoma Breast of Women

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Abstract

Previous studies have referred that the breast cancer, with particular histological traits, can be affected by the reproductive factors. In the current study, we employed 30 breast cancer cases for women in age (15-55 years) to study how factors such as microscopic screening, gross examination, as well as the age, can impact on histological type and grade of tumor. After the organ eradication from the patients, the tissue samples were picked, as fast as possible, and placed in 10% formalin directly. In such a way, we maintained the cells and cellular components in a situation that can be described as similar as possible to the living cells and preserved the antigenicity in order to be processed without any modification. Samples were divided depending on various factors, such as anatomical location, the nodal status, and tumor relationship to surgical margin, weight, size, and age. Each sample was sectioned and fixed onto a private embedding cassette to keep the tissue treatment. The histochemical stains, which is include the eosin and hematoxylin, had utilized to afford a disparity to tissue sections. We found that the majority of the 30 women with breast cancer were at age (31-40 years) and that 12 patients of them were involved left breast, while the other 18 patients included right breast. In agreement with previous studies that have investigated the level of risk of histological subgroups, our study concludes that the link between breast cancer risks and reproductive factors is vary depending on the histological structure of the tumor.

Keywords: Breast carcinoma; breast neoplasms; cancer measurement; cancer staging

Introduction

One of the most important indicators for survival percentage for women with breast cancer is the tumor size and its correlation with lymph node developments (¹). Depending on the cancer staging manual AJCC/UICC (American Joint Committee on Cancer/Union for International Cancer Control), the size of the tumor can be determined (²). Among the different types of cancer that may occur in women around the world generally, breast cancer can be considered as one of the most popular, and it can be diagnosed in one of three women with cancer. After age 40, the percentage of the incidence of breast cancer will increase, while after age 50, the percentage of the incidence will be the highest and reach about 80% of invasive cases. For the cancer diagnosis, the pathologists implement several procedures including the estimating of breast cancer tumor morphology, where it will subject to the screening by the optical microscope, and the degree of differentiation, where it will point the similarity between the breast cancer tumor morphology and the extent of its identity with the other healthy tissue of the organ. The outcomes of such steps will definitely lead to the breast cancer diagnosis (³). Nevertheless, the tumor stage is deal with as an uncertain indicator because of its low level reproducibility (⁴). Furthermore, the bad inter- and intra-observer approval, can affect the optical specific morphology, which leads to non-utilize the grade as an essential indicator for the results. During the specific tumor determination, the pathologist may determine various grades to the same tumor after repeat the assessment for other times (⁵).

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Risk factors for Breast Cancer:

In wealthy societies with the western lifestyle, where people obtain diets that rich-fat and protein, and high caloric, concurrently with the absence of physical exercise, breast cancer can exist in high percentage. The affluent societies like Europe, North America, and Australia, with such a kind of lifestyle, have reached the top of the incidence percentage with (70-90) newly cases for every 100,000 people per year. On the other hand, societies, which have become rich and industrialized recently, have shown a significant increase in disease incidence rates and mortality. However, the main risk factors that have determined involve the dietary and obesity, hereditary, gynecological, age, lifestyle which include the smoking, alcohol and also exercise, oxygen reactive types, as well as the radiation dose and the factors of the environmental pollutants. While breast cancer incidence increases obviously up to the age (50 years), the incidence rate tends to raise in slow level after the age (50 years) (6). Further, the smoker women might reach menopause early compared with nonsmoker women (7), while the percentage of alcoholic women, whose have the risk of evolving breast cancer, might reach to 15% (8).

In general, there is a link between the breast cancer and the level of endogenous estrogen. When the level of estrogen in the serum or urine is high, and the level of SHBG (sex hormone binding protein) is low, this can lead to high abundance of free estradiol. In turn, such a bioavailability leads to increasing the risk of breast cancer caused by endogenous and exogenous estrogens (9). Specific pollutants called xeno-estrogens, which involve the food preservatives, dyes, pesticides, and other, can play an important role in the causes of breast cancer, as long as they intervene with factors like the action of endogenous estrogens (10), the benign of breast cancer history (6), and the Ionizing radiation (11).

Materials and Methods

Our study population was included 30 female, at age (15-55 years), had diagnosed with breast cancer. Factors such as the family history with breast cancer, age, as well as other immunology disease, had been employed for current study. Such a research can lead to a great prospective study about the cancer risks and reproductive factors (12). The survey about the medical family history was based on whether the mother or sisters has diagnosed with a breast cancer, and the existence or lack of cancer (13). After the organ eradication from the patients, the tissue samples were picked, as fast as possible, and for the fixation, it placed in 10% formalin directly. After the fixation step, samples were trimmed by utilizing special scalpel to make them suitable for the labelled tissue cassette. For removing the fixation, and also the water, from the tissues, samples were passed through alcohols with graded concentrations (70 - 95 - 100%). Then, the paraffin block that contain the tissue was trimmed to reveal the interest tissue, and cut into fine sections with about 5 micrometers diameter by microtome machine. Both types of the histochemical stains, the eosin and hematoxylin, were typically used for the tissue sections contrast, to improve the tissue structures and make it clearer and easy to estimate.

Results and Discussion

![Figure - 1: Age Distribution of Breast Cancer (Female No.)](image-url)
Age Distribution of breast cancer (female %)

In the current study, the majority of the 30 women with breast cancer were at age (31-40 years) (Figure 2). The mean age was about (35.26) years, and about 12 patients of them were involved left breast, while the other 18 patients included right breast.

One of the most widespread cancer that involves the female’s population around the world is the breast cancer. In developing countries, its incidence percentage has raised at rate 3-4%, and generally diagnosed at final stages (9). In Nepal, a previous study had been indicated that among the reproductive cancers, breast cancer had the highest prevalence, and the female age category that most exposed was (30-40 years) (15). Our results proved that the female age category (15-50 years) had the highest rate of breast cancer in compared with other age categories. In contrast, previous study had shown that the most popular age category in diagnosis the breast cancer was (41-55 years) (16).

Histological and morphological change of female patient of breast cancer

Fig3: Location of Biopsy: right breast nodule. Age of female 35 years. Gross examination: Multiple pieces of tissue measure (5x4) cm, brown in color, and firm in consistency, cut section reveal white homogeneous area, multiple pieces taken in five cassettes. Microscopic examination: Section revealed hyalinized stroma in broad papillary fibro vascular cores with presence of two cell types epithelial and myoepithelial with normochromic often-oval epithelial cell nuclei and scant mitotic activity Picture of intraductal papilloma.
Fig 4: Site of biopsy: Right breast. Age 18 years. Gross examination: Single piece of tissue measure (4x2x1.5 cm), white in color, and firm in consistency, cut section revealed homogenous, white surface, multiple pieces taken in three cassettes. Microscopic examination: Section revealed pieces of breast tissue showing dilated small duct with intact epithelial lining surrounded by mild fibrosis with area of fibro-adenomatous changes.

Picture of fibrocystic disease with fibro-adenomatous changes.

Fig 5: Site of biopsy: right breast lump. Age 55 years. Gross examination: Four core of tissue measure (1x0.5x0.2) cm, all taken (each two cores) in one cassette. Microscopic examination: Section reveal four core of breast tissue showing increase proliferation of atypical pleomorphic ductal cell, arranged in a ducts, cords and single cells, on a background of desmoplastic stroma, associated with focal area of lymphocyte infiltration. Picture of invasive ductal carcinoma.
Fig6: Site of biopsy: left breast mass. Age 35 years. Gross examination: Multiple piece of tissue measure (1x1) cm, all taken in one cassette. Microscopic examination: Section reveal marked infiltration malignant neoplastic cell showed marked necrosis and stromal invasion, few cluster showed marked stromal invasion. Picture of non-differentiated invasive ductal carcinoma grade 2.

Depending on (modified Scarff-Bloom-Richardson grade), which is basically grading system, parameters such as the nuclear size and pleomorphism, the level of tubule formation, and mitotic rate had been measured. Each factor was specified with a score (1-3), however, the final grade was determined from the total of every single scores. Regardes to the differentiation degree, (grade I) is a well differentiated with scores (3-5), (grade II) is a moderately differentiated with scores (6-7), and (grade III) is a poorly differentiated with scores (8-9) (17).

Conclusion

Our results showed that the link between the risk of breast cancer and the reproductive factors is vary depending on the histological pattern of the tumor.

Although not included it in staging guidelines because of the nature of the assessment procedure, the histological grade of breast cancer considers as a substantial prognostic factor.

Despite the fact that the highest frequency of medullary tumors is existed among multiparous women, it’s considered as not preferred factor effect for pregnancy on the histological type of breast cancer

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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3. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer I. The value of histological


Isolation and Molecular Identification of *proteus mirabilis*
Isolated from Hospitals in the Capital *Baghdad*

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¹Post graduate, Prof., College of Science for Women / University of Babylon, Iraq

Abstract

*Proteus mirabilis* a Gram-negative bacteria belonging the *Enterobacteriaceae* family and that causes several infections to patients such as urinary tract infections, middle ear infections, wounds and burns, in addition to other infections. This study aimed to isolate and diagnose *Proteus mirabilis* bacteria collected from different samples of infection sources from patients present in many hospitals using traditional diagnostic like morphological and biochemical tests as well as molecular methods. The Identification system for Gram negative bacteria GN24 KIT had used to detect *Proteus mirabilis* species . The results of using this kit were confirmed by the molecular diagnosis of bacteria through the PCR technique of the 16S rRNA gene, where the confirmation rate was 100%. The PCR sample was analyzed in Macrogen Corporation–Korea for Sanger sequencing using ABI3730XL, an automated DNA sequences. the findings were obtained and analyzed via genius software in order to Synthesis of the phylogenetic tree of the isolated strains that resulted from the discovery of a new global strain of *Proteus mirabilis* bacteria and was recorded in NCBI genebank with accession number (MN 700085) and name (M.K.84 ).

**Keywords:** Health; Genebank; toxicity; sequencing; *proteus mirabilis*

Introduction

*Proteus* spp . considered one of Gram-negative bacteria and they are members of the family *Enterobacteriaceae*, as well as belong to gastrointestinal microorganisms [1]. *Proteus mirabilis* is a rod formed bacteria, it is commonly documented by its urease generation and recognizable capability to differentiate to elongated swarm cells and the distinctive bull-eye style of motility on agar media plates [2]: *P. mirabilis* return to the Gammaproteobacteria class, and had been for a long time regarded as an individual of the *Enterobacteriales* Order, *Enterobacteriaceae* family, Besides that, lately, one group suggested re-classification of the *Enterobacteriales* order to put *Proteus* in a new position within the *Morganellaceae* family based on the recreating phylogenetic tree depending on four multilocus sequence analysis proteins, ribosomal proteins , shared core proteins [3]. *Proteus mirabilis* was of medicinal significance and is typically responsible for most serious bacterial infections in hospitals including such urinary tract infections, wound, ear infections and other infections [4]. In healthy hosts *P. mirabilis* is not considered a major cause of urinary tract infection , on the contrary , *P. mirabilis* is detected in complicated UTIs comparatively repeatedly like Patients with functional or physiological disorders, in particular patients with urolithiasis or chronic urinary catheter [5]. *Proteus mirabilis* ureolytic activity in the catheterized urinary tract results in ammonia production and an elevation of urinary pH. Under these alkaline environment, magnesium ammonium phosphate (struvite) and calcium phosphate (hydroxyapatite) crystals are usually begin to form by normally soluble urine constituents precipitation [6].

16S rRNA genes encoding a small subunit of rRNA in prokaryotes have been widely used in taxonomic classification and determination of phylogenetic relationships. The 16S rRNA gene sequence is used to detect bacterial species in natural specimens and to
establish phylogenetic relationships between them. This is made possible by the fact that all bacterial species contain the 16S rRNA gene, which has highly conserved regions on which to design universal primers, as well as hypervariable regions that are useful in distinguishing species. The 16S rRNA gene has hypervariable regions which are an indication of divergence over evolutionary time[7]. For addition reasons the use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy was by far the most common genetic marker used, these reasons include (i) its existence in almost all bacteria, often identified as a multigene family or operons; (ii) the function of the 16S rRNA gene has not changed over time, indicating that alterations in the sequence are a more accurate measure and (iii) the 16S rRNA gene (1500 bp) is fairly large for informatics purposes[8].

Genotypic bacterial analysis starts with the PCR product’s nucleotide sequence analysis of the respective gene(s) followed by a comparison of such sequences with the identified sequences in the database [9].

**Materials and Methods**

One hundred and fifty swab samples were collected from different sources of infections (urinary tract infections, Otitis media swab, Stool , Wound swab, Vaginal swab) that were taken from patients suffering from various diseases, which were eighty samples from females and seventy samples of males (With age range from six months to seventy years) present to the several Iraqi capital Baghdad hospitals for the period from the first of September 2019 to January 2020 . Each specimen is immediately inoculated onto the MacConkey’s agar plates where it is considered a selective and differentiation agar which develops only gram-negative bacterial organisms; it could also distinguish gram-negative species on the basis of the lactose fermentation , the selective and differentiating property of MacConkey agar that allows use in both scientific and clinical applications [10]. On the other hand, to see the phenomenon of swarming more clearly , samples were cultured on blood agar plates which was the enriched product often used for cultivating fastidious microbes and the differentiation of bacteria depending on their hemolytic effects [11].

**Identification system for Gram negative bacteria GN24 KIT**

GN 24 is a standard recognition method for Gram negative bacteria common species . The test is dependent on 24-29 miniaturized biochemical tests and on the Internet database. Kit GN 24 consists of 24 wells microtiteration plate strip in the classic 96 well format containing dehydrated substrates Reconstitution of substrates is performed by bacterial suspension inoculation. During incubation, color changes occur in wells due to microorganisms ' metabolic activity. Test results may be measured either by automated readers or visually depending on the color scheme, or by the color definition displayed in the flyer. The results of recognition can be collected from the evaluation table or by using evaluation tools located at (www.diagnostics. sk/idmicro).

**DNA Extraction from Gram Negative bacteria**

According to the ABIOpure (USA) Extraction method , genomic DNA was extracted from bacterial growth.

**Quantitation of DNA**

Quantus Florometer (Promega, USA) has been used to measure the concentration of sample DNA collected for downstream applications.

**Detection of 16S rRNA Genes by Polymerase Chain Reaction (PCR)**

Bacterial 16S rRNA had amplified via specific primer pair designed in Macrogen Company ( Korea ). ( F: 5' - AGAGTTTGATCCTGGCTCAG-3') and ( R: 5'- TACGGTTACCTTGTTACGACTT-3') The PCR condition was: Initial denaturation at 95°C for 5 min , followed by 30 cycles of denaturation at 95 °C for 30s, annealing at 60 °C for 30s and extinction at 72 °C for 1min. Final extension at 72 °C for 7 min , finally hold at 10 °C for 10 min.

**Standard Sequencing**

The PCR amplicon samples was analyzed in Macrogen Corporation laboraty–Korea for Sanger sequencing using ABI3730XL, an automated DNA sequences . the findings were obtained and analysed Using genious software .
Results and Discussion

Of the 150 clinical samples, 110 gives positive results, including 19 (12.7%) isolates belonging to *Proteus mirabilis*, whereas the remaining 91 (60.7%) isolates belonged to other bacteria species as seen in the table (1).

<table>
<thead>
<tr>
<th>Samples No.</th>
<th>Positive culture</th>
<th>Other bacteria</th>
<th>Negative culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>19</td>
<td>91</td>
<td>40</td>
</tr>
<tr>
<td>100%</td>
<td>12.7%</td>
<td>60.7%</td>
<td>26.6%</td>
</tr>
</tbody>
</table>

The outcome of the present study matched Abbas et al 2015 that found the *P. mirabilis* rate (12.6%) of one hundred and thirty-five specimens from patients with various clinical cases[12]. EL-Baghdady et al. (2009) got the results comparable to the present study, which had obtain to isolate *P. mirabilis* at rate reaching to (10%) [13]. Mishra et al. (2001) isolated *P. mirabilis* from different sources of infections at a percentage of 78.3%[14]. Furthermore, AL-Jumaa (2011) isolated just (7%) of *P. mirabilis* from the total of 100 samples obtained from various clinical samples of urine, blood, otitis media, burn, wound and perianus [15].

### Proteus mirabilis Identification

*Proteus mirabilis* identification was carried out according to [16] based on the Colonial morphology (Shape, swarming, odor on MacConkey) and Microscopic analysis involving bacterial cell morphology was examined by Gram-Stain to determine the shape, cell arrangement, and form of Gram-Stain reaction. After staining, different biochemical tests are carried out for each isolate includes Catalase (+ ve) and Oxidase (-ve) In addition to use Identification system for Gram negative bacteria (GN24 KIT) which used as previously mentioned. Its results are shown in Figure (1) below.

![Figure 1. Diagnostic Results to one of *Proteus mirabilis* isolates Using GN24 kit Software.](image-url)
PCR identification

The results of PCR identification of (15 Isolates) *P. mirabilis* using 16S rRNA showed all isolates were *P. mirabilis* and gave a good confirmative identification as shown in the figure (2). All *P. mirabilis* isolates yielded the same band size (1500 bp) which was the product size of primers used for identification.

![Image of PCR results](image)

**Figure 2.** Results of the amplification of 16S rRNA gene of *Proteus mirabilis* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker.

The present research showed that 16S rRNA was a strong selective power to determine isolated *P. mirabilis*, which was compatible with the Saleh et al. (2019) 16S rRNA test to recognize *Proteus* spp. isolated from urinary tract infection patients and the Mukhtar et al. (2018) finding the 16s rRNA gene used to identify *Proteus mirabilis* on banknotes, the two above research have been reported to be a strongly selective force for *Proteus* spp recognition by 16S rRNA [9,17].

The 16S rRNA gene, which has a length of 1.5 kilobase (kb), has proved to be a valuable molecular target because it is found in all bacteria, whether as a single copy or in multiple copies, and is strongly conserved in a species over time.[18] [19].

**Sequencing and Phylogenetic of 16S rRNA gene**

The results of the analysis of gene sequence of the present study showed a match in the sequence of the nitrogenous bases of the 16S rRNA gene of *P. mirabilis* local isolates with *P. mirabilis* global isolates which saved in (NCBI-Genebank), Where the proportion of identical of fourteen isolates were (100%) and the identical ratio of last isolate was (99.63%) , as the result of the current study was close to what Jian-ke et al.,(2015) reached where he was found that the 16S rRNA gene for the diagnosis of *P.mirabilis* bacteria is more than (99%) identical when analyzed at (NCBI-BLAST) site . The same researcher also found that the Phylogenetic tree analysis for 16S rRNA gene of 20 global *P.mirabilis* strains had very high identical ratio in the gene-bank and the match rate was (98.9-99.7%) this ratio was Close to the result of the phylogenetic tree analysis for the current study for the 16S rRNA gene as shown in figure 3 below . Where the results showed in the current study that there was a group of strains that were 100% identical with each other, and these are the following samples (10-14-16-27-29-30-33) these strains differ with some strains in the gene bank in the rate of (6*10^-5) . The other group includes a group of strains that are 100% identical with each other, as well as other strains in the genebank at 100%. an example of this was
the numbered strain (AOUC-001) which matching with samples (20-21-23-28-32), While the sample No. (17) was identical to the gene bank sample (TL14(1)) at the rate of (100%) and differs from the other local and global strains above in ratio about (1.84*10^{-4} 10^{-4}) also, the sample (31) was 100% identical to the sample (ALK042) on the other hand, it differs from the first group strains above by (6*10^{-5} 10^{-5}). Finally, with regard to sample No. (7), it did not match with any other strain (100%), whether they were local or global strains, and the percentage of difference with all strains mentioned previously was (0.002). It will be discussed more broadly in the next topic.

Figure 3. The Phylogenetic tree of 16S rRNA gene for fifteen strains of P. mirabilis bacteria included in the current study in addition to a number of strains of the same species of bacteria from the strains stored in the gene-bank database of NCBI.

**Detect novel strain**

After the 16S rRNA nucleotide sequences were scanned for similarity sequences using online NCBI-BLAST analysis one of the isolates, was discovered which did not match any strain, at 100% of the strains stored in a NCBI-Genebank. The above-mentioned isolation registration was approved by the (NCBI-Genebank) as a new global strain and it was given an accession number (MN 700085). The 16S rRNA nucleotide sequences of new strain were scanned for similarity sequences using
online BLAST to create phylogenetic tree as appear in figure (4). All isolates retrieved from the NCBI GenBank subjected to the analysis of multiple sequence alignment using genius software.

Figure 4. phylogenetic tree of 16S rRNA nucleotide sequences of new strain *Proteus mirabilis* strain M.K.84 accession number (MN 700085).

Through the phylogenetic tree shown in the figure above, we divide the strains into two groups for the purpose of comparison among them in the first group the results show that the numbered strain (EF091150) was matching with strain (CP048787). The two strains differ with the strain that had accession number (LC382139) in a ratio (5.93 × 10⁻⁴). In the second group the strain which had accession number (CP029133) was matching with strain (AM942759). The two strains also differ with the strain that had accession number (CP042907) in a ratio (5.93 × 10⁻⁴). The rate of difference between the strains of the first group and the second group was (0.0016). The end result of the new strain (MN 700085) was It differs with the strain of both groups in ratio (0.0022). The nucleotides substitutions that appeared in the new strain (MN 700085) was shown in Figure. 5.
Figures 5. Consensus identity of new strain (MN 700085) sequence after analyzing it in genius software.

During the analysis of the sequence of the new strain in the genius program, the result was a match with the strain of accession number (CP042907) at (99.63%) and by five nucleotides in the form of substitutions at the sequences (88-186-245-919-945) (C-T: C-G: T-G: C-T: T-G).

Acknowledgments

We gratefully thank University of Babylon, Science college for girls for assistance and permitting to performed this work in (microbiology Lab), Advance Scientific Office molecular lab (ASCO.Lab) and the following institutions crew (Al Kindy Teaching Hospital, Central Teaching Hospital of Pediatrics, Ghazi al-Hariri hospital for surgery specialist, Al- Sader Hospital, Teaching Laboratories Institute) for supporting and supplying the clinical samples for this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.
Conflict of Interest: None

Funding: Self-funding

References
Diabetes and COVID-19 Pandemic: A Potential Mechanisms: A Review

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Abstract

Diabetes is the greatest public condition among patients with coronavirus disease and has been found to affect prognosis worldwide. It is revealed that hyperglycemia is one of the elements that elevates the risk of consequences in these persons. This study designed to analysis the overall features of the novel coronavirus and provide an understanding of the coronavirus disease in diabetic patients, and its treatment.

These patients are usually treated with various medications and this review clarify the role of metformin and dipeptidyl peptidase 4 inhibitors as helpful factors in these patients. Recommendations are made on the probable pathophysiological mechanisms of the association between coronavirus and diabetes, and its management. Additional study about this association and its clinical managing is necessary.

Keywords: Diabetes mellitus, Coronavirus disease, Severe acute respiratory syndrome-coronavirus-2, Metformin, Dipeptidyl peptidase 4 inhibitors.

Introduction

Diabetes mellitus (DM) is a main cause of morbidity and mortality through the world. It is related with various micro- and macrovascular complications, that eventually influence the general patient’s endurance (¹).

An association between infection and diabetes has long been clinically known (²). Current epidemiological data of the Italian reported as the risk of mortality in coronavirus (COVID-19) patients progressively increases with the numbers of the comorbidities (³).

Coronavirus belong to the Coronaviridae, the members of which infect a wide-ranging range of hosts, generating symptoms and diseases ranging from a common cold to severe and eventually fatal diseases as severe acute respiratory syndrome (SARS), Middle-East Respiratory Syndrome (MERS), and as of present COVID-19 (Figure 1) (⁴).

Differential Diagnosis of COVID-19:

The symptoms of the primary phases of the disease are non-specific. Differential diagnosis must contain the probability of a varied types of respiratory disorders.

- Influenza
- Parainfluenza
- Rhinovirus (common cold)
- Adenovirus
- Human metapneumovirus (HmPV)
- Respiratory syncytial virus (RSV)

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Demography and Clinical Characteristics:

Although all age groups have been exaggerated by COVID-19, the middle age seems between 47–59 years, and commonly greater among severe cases and non-survivors. Signs and symptoms of COVID-19 are illustrated in table 1. It has been shown that males would be poorer exaggerated than females, with a male majority in recent researches. Less cases have been recognized among children and infants, while older COVID-19 cases were noticed to have a poor prognosis (5). Additionally, metabolic syndrome found to be related with a worse scenario in COVID-19 persons. Hypertension is related with worse consequences. These primary clinical explanations that cases with severe COVID-19 are: elderly persons, male, hypertensive, with higher blood glucose concentrations and abnormal liver blood examinations increase the prospect that insulin resistance (IR) might show a significant role in intermediating disease severity. Recent documents from China expected mortality rates near 10% in DM, from a model of 72,314 established cases (6).

Table 1. Signs and symptoms of COVID-19

| Fever       | cough | fatigue | sputum production | shortness of breath |

Review has been documented a considerable correlation between DM and COVID-19 severity (OR 2.67, 95% CI 1.91–3.74). Diabetes and race both seem to be further risk elements emerging in the clinical consequences of the COVID-19 pandemic. Within the hospital population COVID-19, infected persons with DM is overrepresented. (7).

Criteria for Suspicion and Testing:

The diagnosis of COVID-19 is approved by microbiologic analysis. Patients who undergo coronavirus according to WHO criteria must be testing for SARS-CoV-2, also testing for additional respiratory pathogens (e.g., influenza, respiratory syncytial virus, etc) (8).

Glycemic Control and COVID-19:

Limited data are concerning glucose metabolism and progress of acute complications of DM in COVID-19
Patients with infection of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and DM probably initiate higher stress circumstances, with higher release of hyperglycemic hormones levels as catecholamines and glucocorticoids, leading to elevated blood glucose concentrations (9).

Glycated hemoglobin (HbA1c) is considered for estimating blood glucose concentration within 120 days before the test, and higher HbA1c value is associated with the complications risk among diabetics (10). Patients with COVID-19 who has higher HbA1c value may reveal moderately greater level of severity. Moreover, the infection may leading to a rise in HbA1c value (11).

**Inflammatory Markers and COVID-19:**

It was reported that inflammatory markers such as C-reactive protein (CRP) level, serum ferritin level, erythrocyte sedimentation rate (ESR) and clotting factors were positively associated with HbA1c value in cases of COVID-19. Previous data have showed that DM not only leads to dysfunction of the epithelium in the pulmonary cilia and improves the permeability of the vascular system, but also donates dysregulated immune system function (12, 13).

The potential mechanisms of COVID-19 affecting glucose metabolism comprise β-cell damage and IR. Preceding data have documented that certain viruses can cause pancreatic β-cell damage directly (14, 15), and angiotensin converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor has greater expression in pancreatic endocrine tissues than in exocrine tissues (16).

Hyperglycemia and IR lead to improved synthesis of pro-inflammatory cytokines, oxidative stress (OS), and advanced glycation end products (AGEs) as well as stimulating the production of adhesion molecules, (Table 2) that mediate tissue inflammation (17).

| Table 2. Factors that affecting on some process in human (17) |
|---------------------------------|-----------------|
| Factors                        | Effects         |
| - Hyperglycemia                | Pro-inflammatory cytokines |
| - IR                           | AGEs            |
|                                | OS              |
|                                | Production of adhesion molecules |

This inflammatory process may initiate the primary mechanism that leads to a greater tendency to infections, with poor consequences in diabetics. Autopsy revealed that though some of pancreatic cells were deteriorated in pancreatic tissue, whereas immunohistochemical examination and polymerase chain reaction (PCR) tests did not identify the occurrence of SARS-CoV-2 in pancreatic cells (18). COVID-19 patients frequently show on admission lymphocytopenia, and to a reduced extent thrombocytopenia and leukopenia, which are more noticeable in cases with severe disease (19).

A further mechanisms, independent of ACE2 expression, are possible to donate to the additional severe phenotype related with DM in COVID-19. A cytokine storm has been concerned in the multi-organ failure related with COVID-19 and there is good indication from animal models of MERS that DM modifies the cytokine profile and augments a dysregulated immune response which aggravates lung pathology. Hence, higher levels of proinflammatory cytokines, including interleukin-6 (IL-6) and CRP, in addition to higher coagulation activity factor, (Figure 2) noticeable by elevated levels of d-dimer, were also related with severity (20).

The concentrations of these elements can be diminished by lifestyle-related variations. There is rare documents on diabetic ketoacidosis (DKA) with COVID-19 infection. It has been reported cases of DKA precipitated by COVID-19 in a newly diagnosed patient with DM. The diabetic ketoacidosis occurs as
a consequence of insulin insufficiency and improved regulatory responses, which favor the production of ketones (21).

The relationship between SARS-CoV-2 and the reninangiotensin-aldosterone system (RAAS) might reveal additional mechanism in the pathophysiology of DKA. Moreover, in T2DM an inequity between coagulation and fibrinolysis happens, with higher concentrations of clotting factors and inhibition of the fibrinolytic system. These irregularities favor the progress of a hypercoagulable pro-thrombotic state (22).

Figure 2. COVID-19 in diabetic patients (22)

It has been thought that the measure of clinical (skin tags, acanthosis nigricans, body weight, body mass index, waist/hip ratio) and biochemical (fasting glucose, insulin, leptin, adiponectin, leptin/adiponectin ratio) parameters related with IR, to conclude if they were related with COVID-19 severity. Consequently, large cohorts of prospectively genotyped cases might recognize genetic polymorphisms related with COVID-19 occurrence or severity that would improve the understanding of the mechanistic basis for the difference in severity of the infection.

The record current publication, encompassing 7336 patients, revealed the risk of fatal result from COVID-19 was up to 50% greater in diabetic patients. Additionally, well regulated blood glucose was related with lesser hospital mortality than uncontrolled glucose (hazard ratio 0.14) (23).

Renin Angiotensin System and COVID-19:

The ACE2 is a carboxypeptidase that is mainly located in the membrane. It is homologous to ACE (24). The ACE2 down-regulates the RAAS and acts as an inhibitor of angiotensin II (AngII), an active peptide causing pro-inflammation action, vasoconstriction, profibrosis, stimulating aldosterone secretion by binding to the AT1 receptor, converting it into Ang-(1-7), an active peptide with opposite possessions to AngII. It has been revealed that Ang-(1-7), by binding to the Mas
receptor, tempted vasodilatation and revealed anti-fibrosis and anti-inflammatory properties (25) (Figure 3). Also, AngII is inhibited by an aminopeptidase which converts AngII into AngIII, which prompts vasodilatation and elevates natriuresis. ACE2 then converts AngI into Ang-(1-9), which is also converted into Ang-(1-7) by ACE1 (26).

Additionally, SARS-CoV-2 binds to ACE2 which is largely expressed by the intestine, lung, and epithelial cells of blood vessels. The RAAS activity is elevated in the lung and is a major source of circulating AngII due to increased expression of ACE. Lung ACE2 regulates the RAAS activation balance by regulating the AngII / Ang1-7 ratio. AngII pulmonary AngII exacerbates vascular permeability, initiating pulmonary edema (27).

Metformin in COVID-19: A Possible Role

Metformin is deliberated one of the safest oral hypoglycemic agents. It lessens IR, but does not promote insulin secretion from β-cells, and so it is not related with increased risk of hypoglycemia (28).

It has recommended that metformin be used as a medication to combat the virus. Also, women who are taking metformin may be at lesser risk for serious COVID-19. In 6,200 diabetic adults or obese who were hospitalized with COVID-19, there were less deaths among women who had filled their 90-day metformin drugs than among those not taking it. This association was not shown in men. So, it is known that metformin has diverse influences between men and women (29). Metformin prompts AMP-activated protein kinase (AMPK) in hepatocytes by causing its phosphorylation.

This is the primary mechanism by which metformin is responsible for positive effects on glucose and lipid metabolism (30). Since metformin works by activating AMPK, which leads to ACE2 phosphorylation, it can be studied that this hypothetical addition of phosphate group would lead to conformational and functional alterations in the ACE2 receptor (31). This may reduce the binding to the receptor binding domain (RBD) of the SARS-CoV-2 protein (SARS-CoV-2 RBD) due to inactivation by adding bulk PO4-3 molecule. However, when it enters the virus, there is a decrease in the
regulation of ACE2 receptors. This leads to asymmetry of the RAAS which supports the harmful properties of its pro-inflammatory and anti-fibrotic arm, leading to potentially fatal cardiopulmonary consequences. By regulating ACE2, the defect in RAAS can be prevented. Therefore, metformin will not only block SARS-CoV-2 entry but also prevent harmful supplementation by activating ACE2 through AMPK signaling

Conclusions

Present review illustrated that DM is related with severity of the disease and poorer short term consequences comprising death. Stronger individual strategies are advised for diabetics, and more serious investigation and management should be deliberated when they are infected with SARS-CoV-2. It is probable that SARS-CoV-2 may deteriorate pancreatic β-cell function and formation of DKA. High blood glucose and HbA1c level are related with inflammation and hypercoagulability. Though DPP4i have been described to be useful for the long term management in diabetic patients where in DPP4 enzyme is blocked, there is no actual data confirmed on the influence on the prognosis of COVID-19 infection.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflicts of Interest: None

Funding: Self-funding

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The Effect of Training with Rubber Ropes and Controlling the Resistance Arm on the Response Time and some Biomechanical Variables for the Skill of Sending for Wheelchair Tennis Players

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Abstract

Training with rubber ropes is one of the modern training methods that help to develop the absolute strength of specific parts of the body and according to the skill performance, especially when training with different muscle moments that cause the movement of those limbs according to variable resistances, especially for wheelchair tennis players, so the research was applied to a sample of tennis players with chairs. Using these exercises to find out their effect on the serve skill mechanism, this exercises were applied for a period of (10) weeks, on (6) players who are the best players in this competition, and the researcher used foreign and Arab sources and the international information network, observation, experimentation, tests and measurements as means of collecting information.

A video camera for filming and using kinematic analysis to extract the mechanical variables, and the researcher came up with several conclusions, including that training according to the results of the analysis and using the change of resistance arms when training with rubber ropes was effective in developing the speed of the centers of the striking arm parts of the research sample. The exercises led to an improvement in the technical performance interdependence (the research sample), which inevitably affected the achievement of good achievement, as well as in improving the response time of the research sample.

Keywords: Resistance arm, response time and control.

Introduction

Training with rubber ropes is one of the modern training methods that help to develop the absolute strength of specific parts of the body and according to the skill performance, in order to develop the forces that cause the movements of those parts for the various sporting activities whose performance is related to the movements of the upper extremity, such as the skill of ground tennis, including wheelchair tennis. And since the movements of the upper limbs of the body of a disabled person in the game of ground tennis are relied upon in the application of basic skills in this game, which are often rotational movements, the muscular work will be related to the action of the various muscle moments that cause the movement of those limbs, and when the force is exerted by the muscles, this force will work to rotate the associated parts around the joints of the body and whenever the level of muscle strength is large, the movement of these limbs will be as fast as possible, and thus the speed achieved in the limbs will contribute to the overall linear velocity of the body when performing the front and back blow or the serve with tennis, which requires the development of strength by controlling the resistance arm when it is shot on the arm and at different distances from the arm in order to develop the torque of force and develop the speed gain for it, given that the parts of the body contributing to the performance are levers whose movement is related to the presence of force and joints around which these parts must be used in

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the service of the skillful performance for this activity, which requires a careful study of the motor performance And the consequent conditions and biomechanical laws in order to reach the correct technical position to achieve the best achievement, so the importance of research came to focus On the strength training exercises for rubber ropes for wheelchair tennis players, which are related to exercises based on the principle of moments, and to determine the importance of the work of these moments in the development of some biomechanical variables and the speed of response to them.

The types of resistance forces have varied as training momentum, which “are of unequal magnitudes and opposite directions or in one direction in the end that support each other in order for the body to move accordingly and change its speed and direction of movement”, which is imposed on the body simultaneously as (the moments of the force of gravity And resistance force moments when training with added weights, body weight, or rubber ropes ) During skill performance, most coaches and players were interested in strength training with various resistances, which are known as “sports movements that are similar in composition in terms of the combination of kinetic performance of strength and speed”, through the use of the best training methods that play a role in influencing the development of strength, including training rubber ropes of various lengths and resistances in various forms, as well as methods of training this regular force, and special strength according to the principle of moments as one of the effective training methods in this field Which is believed to be in addition to its effectiveness in developing muscle strength and ability when performing arm and trunk angle movements in accordance with the mechanical conditions accompanying performance, it can improve performance. The interest of coaches and players in most developed countries of the world has increased in resistance power training according to the Karbala governorate team, and their number was (6) players. They are the best players in this competition and who are preparing to participate in the Iraq Championship 2020. For their lengths (1.76) with a standard deviation (± 0.05).The researcher used foreign and Arab sources and the international information network. Observation, experimentation, tests and measurements as means of collecting information, as well as using a (2) Japanese-origin Casio video camera with a speed of (120) r / s and a computer Automated (laptop Lap top) Lenovo type, and a height and weight measuring device. And rubber bands of different resistors and lengths.

**Research Methodology**

The use of the experimental approach to suit the nature of the research. The sample of his research consisted of elite wheelchair tennis players from the Karbala governorate team, and their number was (6) players. They are the best players in this competition and who are preparing to participate in the Iraq Championship 2020. For their lengths (1.76) with a standard deviation (± 0.05).The researcher used foreign and Arab sources and the international information network. Observation, experimentation, tests and measurements as means of collecting information, as well as using a (2) Japanese-origin Casio video camera with a speed of (120) r / s and a computer.

Automated (laptop Lap top) Lenovo type, and a height and weight measuring device. And rubber bands of different resistors and lengths.

**Tests and measurements**

**A test of the skill performance of the serve and the pre-filming**

The serve skill test of the search sample was conducted
in accordance with the rules of the International Tennis Federation with the conduct of videography, using a video camera at a speed of 120 p/s, for the purpose of analyzing and extracting the variables for the search, and this camera was placed so that the player’s movement is photographed from the weighted moment to receive the ball (as a preparatory position) For the purpose of hitting the transmitter skill, the camera was 7 m away from the midpoint of the path, to ensure the player’s movement was followed, and the height of the camera lens was (1.55 m) perpendicular to the ground.

Bio-mechanical variables

The following biomechanical variables were measured through direct sample imaging and kinematic analysis during pre and posttests, and they are:

velocity variables

1. The speed of the center of the shoulder, elbow and hand of the striking arm according to the following law:

\[
\text{Angular velocity} = \frac{\text{angular velocity (for the forearm or upper arm)}}{\text{time}}, \text{ then extract the circumferential velocity of the hand and shoulder according to the equation (peripheral velocity) = } \frac{\text{angular velocity (in sector / s)}}{N}
\]

The variables of the two equations were extracted through video kinematic analysis of the forehand and serve skills.

- The ball’s launch velocity was extracted from kinetic analysis.
- Response time: measured with the Nelson Kinetic Response Test.

The Nelson Test for Selective Kinetic Response.

The purpose of the test: to measure the ability to respond and move quickly according to the stimulus test.

Necessary tools

- The tennis court: a flat area free of obstructions with a length of (20 m) and a width of (2 m) within which there are three lines of distance between the line and the other (6.40 m), and the length of the line in the middle (1 m).

An electronic stopwatch, a tape measure-recorder/that calls the names first and records the time of performing the test second. Timer/give start signal with timing.

Performance specifications

The player stands at one end of the middle line facing the timer who is standing at the end of the other end of the line and the player takes the standby position so that the middle line is between the feet, then the player prepares with his chair, and the timer holds the stopwatch with one of his hands and raises it to the top and then quickly moves his arm to the left or the right and at the same time he turns on the clock and when the player responds to the start signal, he tries to move with his chair as quickly as possible in the specified direction to reach the side line that is 6.40 m away from the center line, and when the player crosses the right side line, the timer stops the clock.

Test Instructions

- If a player starts in the wrong direction, the timer continues to run the clock until the player changes direction and reaches the right side line.
- The player is given (6) consecutive attempts between each attempt and the other (20) seconds of rest, with (3) attempts on each side.
- The attempts on each side are randomly selected and sequenced.
- The timer must train the start signal in order to be able to give this signal by the arm and operate the watch at the same time.
- The player must not know the number of attempts required of him to perform in order to reduce the player’s expectation.
- The test should start with the timer displaying the (prepare - start) indication for all attempts.

Recording method: The time for each attempt is calculated to the nearest 1/100 second.

Lab score is the average of the six attempts (total score = total attempts ÷ 6 = 0.00 s).
Pilot study

The researcher conducted an exploratory experiment on (10/12/2019) on the sample members in order to adjust the performance of the devices used and to establish the location of the cameras and their preparation completely and the clarity of the images in them. And to identify the obstacles and errors that accompany the research procedures.

Pre-test and videography

The researcher conducted the pre-test and videotaping on (12/12/2019) at the specialized center of the Sports Talent Center for Tennis in Karbala, the researcher conducted performance tests and videotaping and took the test in accordance with international law.

Exercises applied

Restricted strength training (with rubber bands) was applied at the rate of (3) training units per week for a period of (10) weeks and at (30) units by using rubber ropes, which could contribute to the development of the strength level of the sample members according to mechanical conditions, and the exercises focused on special strength according to The theory of force moment (strength x length of the part of the movement), whether the arm or the torso part, and it was given (3) types of exercises in one training unit and these form types of exercises related to skill, movement of the body and the performance of movements that rise with the presence of resistances, as the intensity of these exercises Ranging from (85-95%) of the maximum intensity of the player according to the type of work performed, and a rest period was given according to the working time to rest between repetitions and (2) two minutes between one group and another, and it was carried out by the method of repetitive and interval training with high intensity and the training time was part of the main section within the training unit ranges between (30-40) minutes and the corrugation was (3: 1). The group was subjected to the direct supervision of the researcher with the help of specialized trainers.

The exercises started on 12/18/2019 and ended on 2/10/2020 ,the researcher conducted the test and videotaping on (2/13/2020) at the Sports Talent Center in Karbala, taking care to provide all the same conditions for the two tests.

Results and Discussed

Table 1. Shows measurements of the velocity variables of sending skill for the sample

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tests</th>
<th>Units</th>
<th>Mean</th>
<th>SD</th>
<th>Mean diff.</th>
<th>SD diff.</th>
<th>(t) value*</th>
<th>Moral level</th>
<th>Type of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>The speed of the center of gravity of the body</td>
<td>Pre</td>
<td>Meter/Sec.</td>
<td>1.23</td>
<td>0.230</td>
<td>.35</td>
<td>0.106</td>
<td>3.3</td>
<td>0.0000</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>1.58</td>
<td>0.240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder center velocity</td>
<td>Pre</td>
<td>Meter/Sec.</td>
<td>2.32</td>
<td>0.55</td>
<td>1.53</td>
<td>0.394</td>
<td>3.88</td>
<td>0.000</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>3.85</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attachment center speed</td>
<td>Pre</td>
<td>Meter/Sec.</td>
<td>6.88</td>
<td>41.95</td>
<td>2.04</td>
<td>0.294</td>
<td>6.94</td>
<td>0.002</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>8.92</td>
<td>21.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand center velocity</td>
<td>Pre</td>
<td>Meter/Sec.</td>
<td>12.12</td>
<td>1.63</td>
<td>2.53</td>
<td>0.837</td>
<td>3.02</td>
<td>0.021</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>14.65</td>
<td>1.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cruising speed</td>
<td>Pre</td>
<td>Meter/Sec.</td>
<td>21.70</td>
<td>0.550</td>
<td>2.04</td>
<td>0.453</td>
<td>4.50</td>
<td>0.000</td>
<td>Sig.</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>23.74</td>
<td>0.630</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significance at ≤ 0.05 significance level.
The aim of the research was to study the effect of strength training with rubber ropes with changing arms of resistance in the development of some biomechanical variables for the skill of sending for tennis wheelchair players. There was an evolution in the values of this velocity for the sample members in the dimensional tests, as the development of velocity, whether for the center of gravity of the body or the centers of the joints of the body, is a result of exerting force and its development, because the speed and its development are related to the speed of body parts (such as the speed of the hip, the speed of the elbow and the hand) with the development of force, The more force there was, the more speed, and this is what appeared to the group members during the post tests in each of the hip velocity, the center of the shoulder axis, the elbow and the hand as well as the speed of the head of the racquet, as the researcher used rubber bands according to the skillful performance to develop the muscle groups working on these joints As well as the development of velocity values for these centers.9

The important factor that helps to achieve the fastest possible speed, both when performing the skill of sending and beyond, is the increase in muscle effectiveness and its ability to effectively extend the working joints, which has been emphasized during exercises in which resistances are used as momentum (resistance) during skill performance, as the extension is often used The complete shoulder joint, the elbow joint and the hip joint while maintaining a high position for (the center of gravity of the body) during the final hitting stage, and the player must issue a large central force to maintain the speed achieved in the racket and the continuation of the speed of the center of gravity of the body and the rest of the body at the speed of the possibility of performing the skill of serving.10 All this helped make the change in the velocity of the body parts accused of performance, which is related to the amount of mass of this body or the mass of the body part and its speed between the grooming and hitting phases, which has to do with the form of pushing the instantaneous force exerted, as the momentary force is the reason for the change in the amount of movement of the body Always.11

Some researchers pointed out that training according to the moment of resistance and the absolute and relative muscle moments it encounters enhances the physical and mental ability of the player to visualize the required movement paths, which focus on taking appropriate positions that increase the angular and linear velocity of his body parts by reducing the radii during the performance to which he is exposed. Each repetition ,12 and these repetitions worked to increase the absolute muscle strength of the muscles working in the performance of these stages and increase the efficiency of their neuromuscular compatibility and at appropriate times between repetitions, and all these exercises led to an improvement in the movement paths, especially at the moment of linking the preparation to strike The performance of the strike in the serving skill influences the correlation of the technical performance of the service, which inevitably affects the achievement of good performance, and there had to be iterations to train these parts of the basic skill in order to achieve the special speed and increase the feeling of effective hitting in order to develop the special mechanical variables for these stages which are linked Certainly of the previous stages, to ensure the achievement of the required speed for the body and the racket after launch. As some studies indicate that there is an effect of training using assistive devices in improving efficiency on mastering skills and achieving movement paths for the center of gravity of the body in varying proportions to achieve the goal of training, in addition to that these methods give a positive opportunity to understand the components of skill performance and the possibility of applying it in practice and thus gives an effective effect on Raising the level of motor performance of skills.13

Some studies have indicated that all the characteristics of strength, speed, thrust and direction that a tennis player needs must be in harmony with the goal of performance and the final hitting stage, as the player can take the appropriate technical and mechanical positions due to its association with compound movements and exert the required force with the trunk and arms in a sequential and smooth manner.14
Table 2. Shows the measurements to respond to members of the sample time

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tests</th>
<th>Units</th>
<th>Mean</th>
<th>SD</th>
<th>Mean diff.</th>
<th>SD diff.</th>
<th>(t) value*</th>
<th>Moral level</th>
<th>Type of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response time</td>
<td>Pre</td>
<td>Sec.</td>
<td>3.86</td>
<td>1.6</td>
<td>1.3</td>
<td>0.287</td>
<td>4.52</td>
<td>0.015</td>
<td>Sig.</td>
</tr>
<tr>
<td>Post</td>
<td>2.56</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significance at ≤ 0.05 significance level.

The researcher attributes the reason for the development of the response time to that the exercises in which the sample members used resistors bound by rubber ropes along with the skillful performance and the use of the accompanying corrective feedback contributed to enhancing the attention to the visual stimulus (temporary arm movement), which contributed to the development of the sense of the field of travel distance of the body, the wheelchair, and parts The other body and I helped the players to achieve them in the appropriate manner for good technical performance in order to achieve the best movement response. The researcher was keen to train the members of the sample to perform training while achieving the best angles in the parts of the body and developing the strengths of them in order to economize the momentary muscle strength and in the shortest possible time according to the skillful performance, which facilitated the application of the instantaneous force correctly, especially when starting the movement of the chair according to the correct direction to achieve the correct response that helps In taking the right and appropriate angles to continue this speed.

The application of a good technique of forces to the parts of the body contributing to performance must be done with a balanced kinematic compatibility and high control, especially when extending the striking arm forward at the start of the serve, which comes simultaneously with the swinging arm backward. This means obtaining the mechanical mode that qualifies the player to perform better and good service. Therefore, the researcher was keen to train the members of the sample and train the working muscles of the parts of the body in order to enhance the instantaneous muscle strength in the least possible time according to the skill performance, which facilitated the correct application of the instantaneous force according to the specificity of the skill performance that expresses the final speed that the player reaches.

The results indicated that the exercises that aimed to deepen the foundations of applying the correct movements and according to its technical path by using the development of muscle strengths in the joints (working) in the shoulders, hips and torso were for the development of the instantaneous rotational force of the shoulders, trunk and arms as they are responsible for the performance of the skill of sending and according to the angles The body and its change in the stages of the serve performance, as it appears that the exercises with ropes according to the change of the arms of the resistance have led to the understanding and assimilation of the research sample the technical matters of the performance stages at the moment of hitting through the apparent improvement in the speed of the centers of the parts of this arm, and this improvement is a result of the strength and torque training of the arms and the torso This helped to overcome the mass of the body and the chair during the performance and what follows it from other technical stages, reaching the final stage of striking properly without a technical error, as the use of resistors with less than the maximum intensity when training leads to an increase in the momentary force and the emphasis on changing the tool blocks gives Positive in the acceleration of body parts and device development. Therefore, all exercises aimed at developing muscle strength and momentum with the technical performance required and appropriate for it. This was also emphasized and followed up through the use of exercises using various training methods, and the correction of these values through training to identify possible errors and prepare the exercises according to the requirements of the results of the analysis.
Conclusions

1. Training according to the results of the analysis and by using the change of resistance arms when training with rubber ropes was influential in developing the speed of the striking arm parts centers of the research sample.

2. The exercises led to an improvement in the technical performance coherence (the research sample), which inevitably affects the achievement of a good achievement.

3. The special exercises affected the improvement in the response time of the research sample.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References


Antioxidant Activity and Phytominerals Study of Some Asteraceae Species Growth in Western of Iraq

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¹Assistant prof., ²Lecturer, College of Education for women, University of Anbar, Iraq

Abstract

The antioxidant efficacy of aqueous extracts of twelve plants of the Asteraceae family, which is from natural flora, was investigated by the method of scavenging free radicals of the compound Diphenylpicrylhydrazyl (DPPH) namely Aster subulatus, Calendula officinalis, Carduus pycnocephalus, Carthamus Oxantea, and Carthamus Oxantea linearis, Launaea nudicaulis, Sonchus maritimus and Sonchus oleraceus, this activity is compared to an inhibition ratio (IC50) half of the maximum inhibitor concentration of 50 of the standard ascorbic acid (vitamin C) antioxidant. the most effective of which was Carthamus oxyacantha, which reached 68.430% and Centaurea pallescens 66.432%, and the lowest effectiveness in, Launaea nudicaulis was 40.430% and Koelpinia linearis was 43.816%. The mineral elements were analyzed in the aerial part of plants: (K, Na, Mg, Ca, and P) using Atomic absorption (Spectrophotometer apparatus 5000 (Atomic) - the results showed that plants contain very good ratios of elements as the best plant in terms of content was Carduus pycnocephalus and Carthamus oxykontha.

Keywords: phytominerals, Asteraceae species; toxicity; biological changes.

Introduction

Antioxidants are chemical compounds that can bind to free radicals without turning into a free root, provided that they are not harmful to the body and are subject to excretion, that is, the body can get rid of them, they work to provide the free electron that binds with the other free electron resulting from the oxidation process, thus reaching The state of stability (1) and with this mechanism, the antioxidants maintain the cell and not change the chemical composition of the basic components of the cell, such as fats, proteins, genetics, etc. They are oxidized, and thus you can delay or prevent the emergence of oxidation significantly and with multiple mechanisms, some of which exist naturally in the body, that is, they are self-forming, and the body of the organism manufactures them in order to protect itself from the impact of oxidation of enzymes and vitamins, such as vitamin A, C, E Secondary metabolites in plants (5) which are found in fruits, vegetables and herbs, examples of which are carotenoids, rotenoids, phenolic compounds and flavonoids that trap free radicals and are more effective than vitamins. Antioxidants are also added to foods to help prevent spoilage, Exposure to oxygen and sunlight are the main factors causing food oxidation. (6) The Asteraceae family is one of the largest families in vascular plants at all, and the majority of this family is either in the form of trees, shrubs or herbs, and it is one of the richest plant families in the world as it has about 1600-1700 genera and 24000 species, Monoecious, leaves alternate or opposite, flowers bisexual, the female radial of various shapes, and the corolla is either ligulate in the tubular flowers in the discus flowers, and the fruit Achenes. This family have an anti-oxidative potential (2) and against pathogenic bacteria and infections (3) and also for cancers, and wild plants possess a good content of mineral elements. (4) The aim of the research is study the antioxidant activity in some wild plants of Asteraceae family and know their content of the main nutrient minerals

Materials and Methods

Plants collection

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The plants were collected during flowering from separate areas in Anbar Governorate, from March to mid-August of 2018, after cleaning the plants, they were naturally dried in shade, taking into account the constant stirring, then they were milled using an electric grinder, and then packed with clean and sterile glass containers.

And sterilized

Measurement of oxidative efficacy

the DPPH method, according to what was stated in. (8)250 mg of aqueous leaf extract (5 mg of crude extract / ml of water) was dissolved, and the volume was supplemented to 10 ml of methanol, to a final concentration of 0.5 mg / ml. Standard solutions were prepared by dissolving 250 mg of Ascorbic acid in 25 ml of distilled water, so that the final concentration was 10 mg / ml, and the latter was considered a storage solution of which four concentrations were prepared from which four concentrations of 0.4, 0.3, 0.2, 0.1 (mg / ml) were considered as a control sample and placed in Tubes with plant solutions one by one. 3 ml of DPPH root was added to all tubes and shaken well in the Voretx apparatus. The tubes were placed in the dark for half an hour. After this period, the absorbance of the solutions was measured at a wavelength of 517 nm compared to the control sample DPPH.

Determination of mineral elements

The concentrations of mineral nutrients (K, Na, Mn, Mg, Ca, and P) were measured using an atomic absorption device (Atomic absorption Spectrophotometer 5000, by weighing 1g samples of the plant powder for the studied species and cooled down, dissolve the ashes in 5 ml of hydrochloric acid (20%), then filter the solution using filter paper, and complete the volume to (50) ml using distilled water.

Results and discussion

Oxidative Activity:

The ability of the aqueous extracts of the studied plants to scavenge free radicals was tested using the compound diphenyl-1-picrylhydrazyl-2,2 DPPH, which was transformed into 1,1-diphenyl-2-picrylhydrazine in a reduced form DPPH –H, where it gave an antioxidant such as ascorbic acid. The hydrogen root of the DPPH reagent leading to a change in coloration. (10) the results of the antioxidant reaction of plants showed that the best DPPH was for Carthamus oxyacantha which reached 68.430%, followed by the value of Centaurea pallescens 66.432%. Comparing this effectiveness with the IC value of ascorbic acid which inhibits 50%, we find that these plants greatly surpassed it, which are among the plants of dry areas, and the lowest value of root scavenging was in Launaea nudicaulis, which amounted to 40.816%, and Table (1) shows the e percentage of antioxidant efficacy of plants compared to ascorbic acid.

Table (1): Antioxidant efficacy of the studied genera, compared with ascorbic acid (LSD 5%=2.649)

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Antioxidant %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aster subulatus</td>
<td>44.430</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>50.321</td>
</tr>
<tr>
<td>Carduus pycnocephalus</td>
<td>58.281</td>
</tr>
<tr>
<td>Carthamus oxyacantha</td>
<td>68.430</td>
</tr>
<tr>
<td>Centaurea bruguierana</td>
<td>63.654</td>
</tr>
<tr>
<td>Centaurea pallescens</td>
<td>66.432</td>
</tr>
<tr>
<td>Koelpinia linearis</td>
<td>43.430</td>
</tr>
</tbody>
</table>
The results obtained from the antioxidant reaction of the aqueous extracts of the studied plants showed high efficacy and great ability to displace free roots, and this is confirmed by the studies on both wild plants having a high ability to heal and increase the body’s immunity, especially the species of the Asteraceae family. These plants possess significant amounts of phenolic acids, as well as flavonoids, tannins and fatty acids, as such compounds are widely distributed in the plant kingdom, especially this family, which are distinguished by their effective properties as antioxidants, as the plant components are the most displacement of the roots. This has been confirmed in many studies. Moreover, the efficacy of plant extracts is relatively related to the content of the active chemical compounds they contain, and this was very clear for each of the plant regions which classified as xerophyte plant.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Antioxidant Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Launaea nudicaulis</td>
<td>40.816</td>
</tr>
<tr>
<td>Sonchus maritimus</td>
<td>49.439</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>47.921</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>49.396</td>
</tr>
</tbody>
</table>

Figure (1): Standard curve for ascorbic acid to find the inhibitory concentration of the oxidative half-activity of the DPPH root.

The content of mineral elements shows Table (2,3,4,5) the content of the studied plants of mineral nutrients. The results showed a high percentage of potassium, sodium and manganese, which work to protect the body from osteoporosis and paralysis, and works to increase fertility, and is useful in treating muscle spasm and that its severe deficiency leads to stop the heart muscle in the state of diastole, as it is useful in cases of mental deficiency and has importance in regulating blood pressure and heartbeat and activating all excretory systems in the body such as the kidneys and colon by exchanging ions with potassium, as well as its importance in excreting CO2 from the body and without it to accumulate CO2 in the blood has become acidic, thus preventing the occurrence of cancer diseases, and
this element has an important role in the representation and transport of sugars inside the plant. (16) and the table shows the presence of phosphorus, as its presence in the plant occurs naturally as a result of its absorption from the soil and is important in Water balance within cells, and it is important for neuromuscular stimulation as it is excreted from the body through the kidneys (14) Calcium has a benefit in preventing osteoporosis and joint pain, and it also has an important role in the blood clotting process as its ions are naturally present in the blood, factor (4) that is involved in the formation of the enzyme that helps convert prothrombin in to thrombin, and the presence of this The element explains the indications for using the plant as a tonic and tonic for the heart muscle (15) And the presence of magnesium, calcium and phosphorous in wild plants, which gives them a role in treatments for morbidity and bone formation, and is useful for muscle contraction, hypertonia, reducing and treating nausea, and gastrointestinal disorders, and this explains the importance of the plant in the treatment of general weakness of the body and anemia to produce Hemoclopin, which increases the body’s resistance, supports the immune system, as well as increases energy production, which the body needs greatly, especially in women and children (6) As for manganese, their percentage was weakness, anemia, and skin pimples as well as being an antioxidant and that these elements have a role in increasing the oxidative activity of plants (4).

Table (2) the values of the mineral elements phosphorous and manganese in the studied genera of Asteraceae family

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Mn ppm</th>
<th>P Ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aster subulatus</td>
<td>120</td>
<td>811</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>152</td>
<td>789</td>
</tr>
<tr>
<td>Carduus pycnocephalus</td>
<td>183</td>
<td>810</td>
</tr>
<tr>
<td>Carthamus oxyacantha</td>
<td>163</td>
<td>503</td>
</tr>
<tr>
<td>Centaurea bruguierana</td>
<td>132</td>
<td>409</td>
</tr>
<tr>
<td>Centaurea pallescens</td>
<td>124</td>
<td>854</td>
</tr>
<tr>
<td>Koelpinia linearis</td>
<td>165</td>
<td>822</td>
</tr>
<tr>
<td>Launaea nudicaulis</td>
<td>201</td>
<td>799</td>
</tr>
<tr>
<td>Sonchus maritimus</td>
<td>254</td>
<td>793</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>176</td>
<td>699</td>
</tr>
<tr>
<td>Picris babylonica</td>
<td>169</td>
<td>496</td>
</tr>
<tr>
<td>Xanthium brasilicum</td>
<td>231</td>
<td>643</td>
</tr>
</tbody>
</table>
Table (3) the values of the metallic element magnesium in the studied genera of the Asteraceae family

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aster subulatus</td>
<td>0.123</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>0.105</td>
</tr>
<tr>
<td>Carduus pycnocephalus</td>
<td>0.176</td>
</tr>
<tr>
<td>Carthamus oxyacantha</td>
<td>0.104</td>
</tr>
<tr>
<td>Centaurea bruguierana</td>
<td>0.120</td>
</tr>
<tr>
<td>Centaurea pallescens</td>
<td>0.122</td>
</tr>
<tr>
<td>Koelpinia linears</td>
<td>0.110</td>
</tr>
<tr>
<td>Launaea nudicaulis</td>
<td>0.151</td>
</tr>
<tr>
<td>Sonchus maritimus</td>
<td>0.114</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>0.190</td>
</tr>
<tr>
<td>Picris babylonica</td>
<td>0.164</td>
</tr>
<tr>
<td>Xanthium brasiliicum</td>
<td>0.120</td>
</tr>
</tbody>
</table>

Table (4) the values of the mineral element calcium in the studied genera of the Asteraceae family

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Ca %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aster subulatus</td>
<td>0.101</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>0.126</td>
</tr>
<tr>
<td>Carduus pycnocephalus</td>
<td>0.120</td>
</tr>
<tr>
<td>Carthamus oxyacantha</td>
<td>0.142</td>
</tr>
<tr>
<td>Centaurea bruguierana</td>
<td>0.128</td>
</tr>
<tr>
<td>Centaurea pallescens</td>
<td>0.103</td>
</tr>
<tr>
<td>Koelpinia linears</td>
<td>0.111</td>
</tr>
<tr>
<td>Launaea nudicaulis</td>
<td>0.113</td>
</tr>
<tr>
<td>Sonchus maritimus</td>
<td>0.176</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>0.123</td>
</tr>
<tr>
<td>Picris babylonica</td>
<td>0.187</td>
</tr>
<tr>
<td>Xanthium brasiliicum</td>
<td>0.199</td>
</tr>
</tbody>
</table>
Table (5) the values of the mineral elements Sodium and Potassium in the studied genera of the Asteraceae family

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Na %</th>
<th>K%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aster subulatus</td>
<td>0.231</td>
<td>0.230</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td>0.298</td>
<td>0.211</td>
</tr>
<tr>
<td>Carduus pycnocephalus</td>
<td>0.276</td>
<td>0.201</td>
</tr>
<tr>
<td>Carthamus oxyacantha</td>
<td>0.234</td>
<td>0.311</td>
</tr>
<tr>
<td>Centaurea bruguierana</td>
<td>0.322</td>
<td>0.318</td>
</tr>
<tr>
<td>Centaurea pallescens</td>
<td>0.344</td>
<td>0.198</td>
</tr>
<tr>
<td>Koelpinia linearis</td>
<td>0.234</td>
<td>0.230</td>
</tr>
<tr>
<td>Launaea nudicaulis</td>
<td>0.210</td>
<td>0.212</td>
</tr>
<tr>
<td>Sonchus maritimus</td>
<td>0.209</td>
<td>0.240</td>
</tr>
<tr>
<td>Sonchus oleraceus</td>
<td>0.291</td>
<td>0.332</td>
</tr>
<tr>
<td>Picris babylonica</td>
<td>0.245</td>
<td>0.309</td>
</tr>
<tr>
<td>Xanthium brasilicum</td>
<td>0.345</td>
<td>0.321</td>
</tr>
</tbody>
</table>

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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The Influence of Cement Spacer Thickness on Retentive Strength of Monolithic Zirconia Crowns Cemented with Different Luting Agents (A Comparative in-vitro Study)

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Abstract

Aims of Study: to evaluate and compare the retentive strength and the failure-modes of monolithic zirconia crowns with different cement spacer thickness, cemented with two different types of luting agents.

Materials and Methods: Forty sound maxillary first premolar teeth were divided into two main groups (n=20): Group A: cement spacer thickness of 80μm; Group B: cement spacer thickness of 120μm. The groups were further subdivided into two subgroups (n=10): (A1, B1) cemented with Panavia F2.0 resin-cement; (A2, B2) cemented with Riva luting plus RMGIC. All the teeth were prepared to receive monolithic zirconia crowns with the following features: (6° convergence angle, 0.8mm deep chamfer finishing line, 4mm axial wall height with planar occlusal reduction). Afterwards, the teeth were scanned and the crowns were fabricated and cemented. Zirconia crowns were pulled-out along its path of insertion using universal testing machine. Failure stresses were calculated in MPa. Failure-modes was assessed using magnifying-lens (2.5X).

Results: The highest mean retentive values (in Mpa) was recorded by subgroup B1 (8.651); followed by subgroup B2 (7.765); subgroup A1 (7.309) and subgroup A2 (6.875). Independent t-test showed a statistically significant difference between A1 and B1 subgroups. Concerning the failure-mode, the majority of samples revealed adhesive failure between teeth and cement.

Conclusion: All the tested crowns have mean retentive values within the clinically acceptable limit, increasing cement space resulted in a significant increase in the retention when Panavia F2.0 used as a luting agent, while, it has no significant effect on the retention when RIVA luting plus was used.

Keywords: cement space, monolithic zirconia, resin-cement, retentive strength, RMGIC.

Introduction

Over the past few years, there has been a remarkable shift towards high-strength, all-ceramic restorations; In-particular those fabricated from zirconium-oxide (ZrO2) ceramics, because of their esthetic, biocompatibility and mechanical properties 1. A common cause of failure of crowns and bridges was reported as a loss of retention 2. Retention form could be defined as the quality of the preparation that prevents the restoration from being dislodged by such forces parallel to its insertion pathway. Retention is dependent on several factors such as form of prepared teeth, roughness of the intaglio surface of the crown restorations, type of cement and its film thickness 3. Furthermore, fitness of crown restorations plays a substantial role in their retention 4. Previously, researchers theorized that better retention could be attained with the frictional fit between the intaglio surface of the restoration and the tooth preparation 5. However,
this theory has been rejected because a perfect fit could not be attained during the cementation, due to the lack of space for the cement. Many researchers have agreed that final fitness of the restoration after cementation would be improved by providing cement spacing during crown fabrication. Consequently, the retention of the cemented crown could also be improved. Cement spacer permits increased space for the luting agent thus reducing stress formed during cementation, resulting in improved fitness and retention of the restoration. Conversely, if the cement space was exceedingly wide, the seated crown will be loose on the preparation; the probability of crown loosening during function would considerably increase, compromising its longevity.

Definitive cementation represents an essential step through the restorative procedure. The precise selection of luting agent and standardized application of cementation protocol are crucial for proper retention and sufficient marginal sealing of the restoration in time.

The objectives of this study were to evaluate and compare the influence of difference in cement spacer thickness on the retentive strength of monolithic zirconia crowns cemented with two different types of luting agents (adhesive resin-cement and resin-modified glass-ionomer cement) and to evaluate the failure-modes of tested crowns.

**Materials and Methods**

Forty sound human maxillary first premolar teeth extracted for orthodontic purpose from patients with the age of 18-23 years had been used in this study, only teeth with two-separated roots were selected to withstand removal from the imbedding-resin during testing, the teeth were cleaned and then disinfected in 1% thymol solution for 48 hours to avoid fungal and bacterial growth. U-shaped notches were made on each root to act as a mechanical mean for retention, each tooth was embedded in specially-fabricated cylindrical rubber-mold containing cold-cure acrylic (Veracril, New Stetic, Colombia) parallel to the long axis to within 2mm apical to the CEJ to simulate the biologic width with the aid of dental surveyor (Paraline, Dentaurum, Germany).

Teeth were randomly divided into two groups (n=20), according to the cement spacer thickness used as: Group A (80μm) and Group B (120μm). They were further subdivided into two subgroups (n=10) according to the type of luting agents: subgroup (A1, B1) cemented with adhesive resin-cement (Panavia F2.0, Kuraray, Japan) and subgroup (A2, B2) cemented with resin-modified glass-ionomer luting cement (RIVA luting plus, SDI, Australia).

Teeth samples were prepared to receive monolithic zirconia crowns (Katana zirconia ML A light, Kuraray, Japan) according to the following criteria: 6-degree total convergence angle, a planar occlusal reduction, 0.8mm deep chamfer finishing line and 4mm axial height from the finishing line to the occlusal level buccally and palatally (Figure 1).

![Figure (1): Finished prepared tooth](image)

A: Occlusal view, B Lateral view.

Samples scanning was performed using powder-free digital scanner (in-Eos X5 in-lab scanner, Sirona, Germany). Measurement of the surface area for the scanned preparation was performed using AutoCAD architecture software. During designing procedure, the zirconia crowns were deliberately received four macro-retention bars to help removal of the crown after cementation, 5-axis milling machine (In-Lab MC X5 milling machine, Sirona, Germany) was used to produce the zirconia crowns.
After completion of sintering procedure, the intaglio surface of each crown was air-abraded with 50μm aluminum-oxide for 15 seconds using 4-bar pressure at 10mm distance and 45° angle (Pneumatic sandblasting unit, Renfert, Germany). Afterwards, the crowns were cleaned in an ultrasonic cleaner for 5 minutes to remove any residue of blasting agent.

For Panavia F2.0 resin-cement subgroups, two coats of zirconia primer (Z-PRIME Plus, Bisco, USA) were applied to the intaglio surface of the restoration and then lightly air-dried for 5 seconds according to manufacturer’s instruction. Equal amounts of primer (ED primer II A and B, Kuraray, Japan) were mixed for 5 seconds and applied to the tooth surface, left for 30 seconds and lightly air-dried for 5 seconds. Equal amounts of the two pastes (one complete turn for each paste) were mixed for 20 seconds according to manufacturer’s instruction, the cement was evenly distributed on the intaglio surface with a pen brush.

For resin-modified glass-ionomer cementation, 37% phosphoric acid gel (Super Etch, SDI, Australia) was applied to the prepared tooth for 5 seconds then rinsed with water and air-dried according to their manufacturer’s instructions. Riva luting plus capsules were mixed for 10 seconds then the cement was injected into the crown.

The cementation procedure for all subgroups was performed using a modified dental surveyor under a constant load of 5-Kg for 5 minutes. The excess cement was removed with a microbrush followed by curing for 20 seconds per surface for resin-cement subgroups according to the manufacturer’s instructions. Thereafter, the samples were stored in deionized water at room temperature for 7 days.

Before the tensile-test, each crown was embedded in a clear cold-cure acrylic-resin (Duracryl® Plus, Kerr, Crech) using a second cylindrical rubber-mold. A stainless-steel screw was incorporated at the top parallel to the long axis of the tooth with the aid of an adapted surveyor. The upper mold was separated from the lower one by 3mm thick sheet of rubber.

After 24 hours, the entire apparatus was mounted in (computer-controlled universal testing machine, Laryee, China) and pulled along the path of insertion in a crosshead speed of 0.5mm/min until failure. The dislodgment force was recorded in (N), divided by the surface area of each preparation in (mm²) to yield the retentive stress in (MPa).

After completion of the test, all samples were examined using a magnifying-lens (2.5X) to assess the failure-modes, according to the classification by Gundogdu and Aladag in 2018 (Table 1).

<table>
<thead>
<tr>
<th>Mode of failure</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Cohesive failure in teeth.</td>
</tr>
<tr>
<td>Type II</td>
<td>Adhesive failure between teeth and cement.</td>
</tr>
<tr>
<td>Type III</td>
<td>Cohesive failure in the cement.</td>
</tr>
<tr>
<td>Type IV</td>
<td>Adhesive failure between the cement and zirconia surface.</td>
</tr>
<tr>
<td>Type V</td>
<td>Cohesive failure in ceramic.</td>
</tr>
</tbody>
</table>

Table 1: Modes of failure as described by Gundogdu and Aladag in 2018.

Statistical analysis of data was performed using SPSS program (Statistical Package for Social Science, IBM Corp., USA).
Results

The descriptive statistics (means, ±SD, Min. and Max.) for each subgroup are presented in (Table 2).

Table 2: Descriptive statistics of retentive strength values of the different subgroups measured in MPa.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Subgroups</th>
<th>N</th>
<th>Mean ±S.D.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10</td>
<td>7.309 ±1.232</td>
<td>5.313</td>
<td>8.999</td>
</tr>
<tr>
<td></td>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10</td>
<td>6.875 ±1.445</td>
<td>4.891</td>
<td>8.706</td>
</tr>
<tr>
<td>B</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10</td>
<td>8.651 ±1.458</td>
<td>5.698</td>
<td>10.597</td>
</tr>
<tr>
<td></td>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10</td>
<td>7.765 ±1.728</td>
<td>4.563</td>
<td>9.632</td>
</tr>
</tbody>
</table>

The highest mean retentive values in MPa was recorded by subgroup B<sub>1</sub> (8.651); followed by subgroup B<sub>2</sub> (7.765); subgroup A<sub>1</sub> (7.309) and subgroup A<sub>2</sub> (6.875) (Figure 2).

![Retentive Strength in MPA](image)

Figure 2: Bar-chart showing the mean retentive strength values for all subgroups in MPa.

Independent samples t-test was used to analyze the data at a level of significance (P≤ .05), the test disclosed a statistically significant difference between B<sub>1</sub> and A<sub>1</sub> subgroups while a statically non-significant difference between the other subgroups has been revealed by the test (Table 3).

Table 3: T-test for comparison of significance between subgroups.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>t-test</th>
<th>Df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;&amp;A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>.722</td>
<td>18</td>
<td>.480* [NS]</td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt;&amp;B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.238</td>
<td>18</td>
<td>.232* [NS]</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;&amp;B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-2.223</td>
<td>18</td>
<td>.039* [S]</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;&amp;B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-1.249</td>
<td>18</td>
<td>.228* [NS]</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
The results for characterization of failure-modes were generally adhesive in nature as illustrated in (Table 4).

### Table (4): Mode of failure of the different subgroups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Subgroups</th>
<th>Type I (%)</th>
<th>Type II (%)</th>
<th>Type III (%)</th>
<th>Type IV (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A1</td>
<td>2 (20%)</td>
<td>5 (50%)</td>
<td>1 (10%)</td>
<td>2 (20%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>1 (10%)</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>B</td>
<td>B1</td>
<td>1 (10%)</td>
<td>8 (80%)</td>
<td>-</td>
<td>1 (10%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>-</td>
<td>5 (50%)</td>
<td>1 (10%)</td>
<td>4 (40%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

**Discussion**

Retention is considered as one of the essential elements which determine the long-term clinical-success of dental prosthesis. There is no single factor on which retention is entirely dependent. In-fact, retention involves a list of factors, all of which have to be taken into account during all stages starting from teeth preparation to the final cementation. Even if a single factor is accidentally neglected it can affect the retention of the crown restorations which further has a direct impact on the longevity of the restoration. Monolithic zirconia crowns were selected for this study due to its superior physical properties that were pliable to the tensile pull-off test.

A total convergence angle of 6-degree was used in this study because it is considered as an ideal convergence angle that improves retention of crown restoration. A planar occlusal reduction was performed because tooth should be prepared in accordance with a clinical simulation. Furthermore, greater surface area would be achieved (when compared to a flat occlusal reduction) thereby increasing its retention.

Because of high flexural strength, zirconia crowns can be cemented either non-adhesively using conventional cements or adhesively with the resin-cement. Therefore, RIVA luting plus RMGIC and Panavia F2.0 resin-cement were selected to represent two distinct groups of luting agents.

The results of this study have been showed that all the tested crowns have mean retentive values exceeding the clinically acceptable limit required to retain crown successfully according to Palacios et al. (2006). In this study we found that 120μm cement spacer thickness has significantly higher mean retentive values than 80μm cement spacer thickness for resin-cement subgroups. It might be explained as when ideal convergence angle would be used for the preparation (6-degree), the expression of the excess cement through this angle would be difficult, this will lead to filtration process causes the cement to separate into particles and liquid phase, allowing the passage of liquid phase while solid particles gathering occlusally which leads to the development of hydrostatic pressure under the crown that increases until it matches the seating force and causes further seating impossible. Increasing space between the prepared tooth surface and the intaglio surface of the restoration, reducing stress areas formed during cementation, and thereby resulting in better fitness and retention of the definitive restoration. This was in accordance with previous studies concerning the increase in the cement spacer thickness.

The results found in this study were inconsistent with that reported by Mehl et al. (2013). However, the different materials of the aforementioned study (titanium implant's abutments, cobalt-chromium crowns and different cementing systems) could be the reason for
Another finding of this study concerning the type of luting agents used, when cement space thickness remains constant, the results showed non-significant difference in the mean retentive stresses for RIVA luting plus RMGIC when compared to resin-cement. This might be due to the chemical adhesion to dentin, improving the retention of RIVA luting plus RMGIC. In addition, the use of phosphoric acid-etching enhanced the micromechanical retention of Riva luting plus RMGIC. Furthermore, the grain particle size of Panavia F2.0 resin-cement (0.04-19μm) is relatively similar to the particle size of the resin-modified glass-ionomer luting cements (≈15μm) which might lead to a relatively similar entanglement or hooking of filler particles to the dentin surface. However, in spite of the non-significant difference, a higher mean retentive values were recorded with Panavia F2.0 resin-cement subgroups than those of RIVA luting plus RMGIC subgroups. The findings of the present study are in agreement with the study by Ernst et al. (2005) and Palacios et al. (2006).

Concerning the failure-modes, the majority of samples for all subgroups showed an adhesive failure (type II and type IV) (52.5% and 25%) respectively. This suggests that the mechanical interlock to air-abraded roughened zirconia surface was greater than mechanical interlock to the tooth and might be also attributed to the application of Z-prime plus which enhances the bond strength of the resin-cement to zirconia crowns. A number of studies had been performed to evaluate the influence of cement space on the retention of cemented crowns, their data produced inconsistent results. A clearly established experimental protocol is not evident from those studies rendering the comparison of results so difficult. Further research is needed to examine the effect of cement spacer thickness on crown retention, various crown material types, different surface treatments, different luting cements, long-term storage, thermocycling.

Conclusions

Within the limitations of this study, the following conclusions were drawn:

1. All the tested crowns have mean retentive values within the clinically acceptable limit required to retain crown successfully.

2. Increasing cement space thickness to 120μm would result in a significant increase in the retention values of zirconia crown restorations when Panavia F2.0 used as a luting agent. While it has no significant effect on the retention of crown restoration when RIVA luting plus was used as a luting agent.

3. The results for characterization of failure-modes were generally adhesive in nature.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

Acknowledgments: Special thanks to Mustansiriyah University/college of dentistry for their guidance and support.

References


7. Eames WB, O’Neal SJ, Monteiro J, Miller C, Roan


HIV/AIDS Status in Baghdad/ Iraq Over Ten Years (2010-2019)

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Abstract

Background: Human immunodeficiency Virus and/or Acquired immunodeficiency Syndrome (HIV/AIDS) hitherto represents a serious global health problem.

Objective: To disclose the status and trend of HIV/AIDS in Baghdad/ Capital of Iraq throughout the last ten years (2010 -2019).

Materials and Methods: A retrospective study of the available data on new HIV cases for the last ten years was conducted in Baghdad/ Capital of Iraq from August 2018 to December 2019. The data included age, sex, annual number of new HIV cases, AIDS-related deaths, years of diagnosis, and risk factors associated-HIV transmission. All suspected HIV/AIDS cases were examined clinically and tested initially for anti-HIV antibodies by ELISA and confirmed by western blot technique.

Results: A cumulative annual number of new HIV cases registered for the last ten years was 287. The trend of number of new HIV cases increased gradually and steadily over successive ten years 2010-2019. The majority of them were males (79.1 %) and adults (≥15 years old) (98.2%). Sexual transmission had a higher rate of new HIV infection (81.9%) mainly through heterosexuals (79.2 %).

Conclusion: Although the trend of the annual number of HIV cases increased with time, Baghdad remains within the area of low HIV prevalence, and there is a potential risk for exposure to an outbreak or epidemic/endemic if no implementation of proper and effective national strategy plans on HIV/AIDS in place. Adult males play a role in rising the risk of illness. Sexual practices mainly through heterosexuals was the major route of HIV transmission.

Keywords: HIV, Status, trend, Toxicity; Baghdad; Sexual transmission

Introduction

HIV/AIDS endures the leading cause of morbidity and mortality throughout the world with a large socio-economic impact. [1]

In 2019, around 38.0 million people in universe living with HIV, with 1.7 million became newly HIV infected , and 700 000 were died from AIDS related illnesses [2-3]. An estimated that 0.7% of the world were infected with new HIV infections, the Middle East region reported the lowest HIV prevalence (<0.1%) while WHO Africa region reported the highest prevalence of HIV (3.7%)[1-4].

Baghdad City is the capital of Iraq with a population of seven million people, of which about four millions are living in Resafa sector and three millions living in Karkh sector. [5]

In Iraq, no information are available on status of HIV/AIDS except one study published in 2007 [6], because of governmental restriction.

Therefore such information are needed to highlight the status and trend of the new HIV cases registered in Baghdad Province/ Capital of Iraq for the period extending from 2010 to 2019.
Materials and methods

This an observational, retrospective study carried out on data of new HIV cases records for Baghdad at National HIV program Center, during 2010-2019. It was conducted in Baghdad City from August 2018 to December 2019. All the cases were referred from doctors working in governmental and private clinics and detected in Central Public Health Laboratory (CPHL)/Baghdad, using ELISA as screening test for HIV and confirmed by Western blot testing. All positive cases are confirmed also by Polymerase Chain Reaction (PCR) and follow up periodically to ensure the effectiveness of anti-retroviral therapy (ARTs) which is free of charge in Iraq.

The detection of the HIV cases is mandatory by Iraqi government for specific groups of people having potential risk for exposure to HIV, such as, suspected HIV patients with their contacts, blood donors, pre-surgical operations, pre-marriage, pre-natal care program.

The data were collected from records and categorized into the years of diagnosis age, sex, and possible routes of HIV transmission.

Ethical approval and permissions were obtained from Ethical Approval Committee of Al-Anbar University in 23/February/2018,N0.24 in coordination with HIV/AIDS Medical Center/MOH.

Data were processed using computer software program of Statistical Package for Social Sciences (SPSS), version 26. For the analysis Chi-square and Yate’s correction test were used, P-value of <0.05 was considered statistically significant.

Results

A cumulative annual number of 287 newly HIV cases were recorded for Baghdad from 2010 - 2019. Their age ranged between 4 - 65 years old. There were 227 males (79.1%) and 60 females (20.9%), giving a male to female ratio of 4.85:1.

Figure 1 shows an increased in the trend of annual number of new HIV cases in Baghdad for both sexes during the period from 2010-2019 with a significantly higher annual number in males than in females (P<0.0001).

Figure 2 shows that the trend of annual number new HIV cases is increased year by year; it was five cases (1.7%) in 2010, reaching to 16 cases (5.6%) for 2015 and 80 cases (27.9%) for 2019, giving grew rate of 6.8% per year. Although there is gradual increasing in the annual number of the cases with time, Baghdad City remained within an area of low prevalence (<0.1%) and the overall annual incidence of HIV infections was <4/100,000. The trend of annual number of alive cases was parallel to the cumulative annual number of new HIV cases with duration of diagnosis while the annual number of HIV-related deaths does not show any clear trend; it remained relatively stable (static) (Figure 2).

Table 1 shows increasing in the annual number of new HIV cases in both sectors of Baghdad City between 2010-2019, with a non-significantly higher percent in Resafa sector (59.9%) compared to Karkh sector (40.1%) (P<0.7091).

Of the total new HIV cases, males (238) were affected more than females (49) (P<0.0001); majority of the males (81.9%) and females (75%) were at age group of ≥25 years old; 17.3% and 21.6% of the males and females, respectively, were aged 15-24 years old. This difference was statistically of significant (P<0.0028).

The distribution of new HIV cases had an upward trend among across all age groups with the least cases recorded among those aged <15 years old (P<0.0027) (Table 2).

Table 3 shows that the possible routes of HIV transmission. It was found that sexual practices was responsible for 81.9% of the cases, blood transfusion for 4.2%, perinatal transmission for 0.35%, using illegal drug in 0.35%, while 13.2% of the new HIV cases denied any risk behavior (no specify or unknown cause). This difference was of highly significant (p-value <0.0001).

Within sexual activity, heterosexual contact constituted 79.2% of the cases followed by bisexual contact at 13.6%, and homosexual contact at 7.2 %. This difference was also statistically of significant (p< 0.01145)
Figure 1 Distribution of annual number of new HIV cases for Baghdad according to gender (2010-2019).

Figure 2 The trend of HIV cases detection (total, alive, and dead) for Baghdad (2010 -2019).
Table 1 Distribution of annual number of new HIV cases in Baghdad sectors (2010-2019)

<table>
<thead>
<tr>
<th>Year</th>
<th>Baghdad Sectors</th>
<th>Karkh</th>
<th>Resafa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>2010</td>
<td>5</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>2011</td>
<td>3</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>2012</td>
<td>7</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>7</td>
<td>2.4</td>
<td>5</td>
</tr>
<tr>
<td>2014</td>
<td>12</td>
<td>4.2</td>
<td>4</td>
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<tr>
<td>2015</td>
<td>16</td>
<td>5.6</td>
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</tr>
<tr>
<td>2016</td>
<td>28</td>
<td>9.8</td>
<td>11</td>
</tr>
<tr>
<td>2017</td>
<td>56</td>
<td>19.5</td>
<td>25</td>
</tr>
<tr>
<td>2018</td>
<td>73</td>
<td>25.4</td>
<td>26</td>
</tr>
<tr>
<td>2019</td>
<td>80</td>
<td>27.9</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>100.0</td>
<td>115</td>
</tr>
</tbody>
</table>

Yate’s X² = 6.304, df=9, P=0.7091

Table 2 Distribution of annual number of new HIV cases for Baghdad (2010-2019), according to age and gender

<table>
<thead>
<tr>
<th>Age group</th>
<th>n = 287</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>0-4</td>
<td>2</td>
<td>0.7</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>5-14</td>
<td>3</td>
<td>1.1</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>15-19</td>
<td>6</td>
<td>2.1</td>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>20-24</td>
<td>46</td>
<td>16.0</td>
<td>35</td>
<td>15.5</td>
</tr>
<tr>
<td>≥ 25</td>
<td>230</td>
<td>80.1</td>
<td>185</td>
<td>81.9</td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>100.0</td>
<td>227</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Yate’s X² = 21.302 df=4, P-value=0.0027

Yate’s X² = 23.508 df=4, P-value=0.0028
Table 3 Possible routes of HIV transmission

<table>
<thead>
<tr>
<th>Route of transmission</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>196</td>
<td>83.4</td>
</tr>
<tr>
<td>Bisexual</td>
<td>32</td>
<td>13.6</td>
</tr>
<tr>
<td>Homosexual</td>
<td>7</td>
<td>3.0</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>12</td>
<td>4.2</td>
</tr>
<tr>
<td>Perinatal</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Illegal drug injection</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>No specify</td>
<td>38</td>
<td>13.2</td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Within sexual route:
\[ X^2 = 269.115, \text{ df}=2, \text{ P-value}=0.0001 \]

Yate’s \[ X^2 = 702.808, \text{ df}=4, \text{ P-value}=0.0001 \]

**Discussion**

In this study, although the curve of new HIV diagnosed cases and alive cases shows clear rising with time from 2010 to 2019, with annual growth rate of 6.8%, and stability (static) of HIV-related deaths, Baghdad is still remaining among the areas with low HIV prevalence (<0.1%), but this may lead to a potential risk for an outbreak or epidemic/endemic of the illness. In comparison, world estimates show that by the end of 2019, the global prevalence of HIV infection was 0.8%, and there was declining in the number of new HIV infection by 23% and AIDS-related deaths by 39% between 2010 and 2019.\,[2,7-8]

Our finding is in the line to that was reported in many countries of Middle East and North of Africa (MENA) region which has the lowest prevalence of HIV globally (<0.1%), increasing in the number of new HIV infections from 2010 to 2019, while HIV-related deaths remained stable and in contrast to Sub-Saharan Africa region which has the highest HIV prevalence of all regions (6.7%)\,[9-11]

In this study, increasing in the new HIV infections with time may be due to increasing in the identification of HIV through an increasing in HIV testing and counselling services, increasing awareness of people about the illness, increasing access for diagnosis and for seeking ART which is free in our country. Besides that, more travel, rapid changes in socio-cultural and religious values. All these factors, collectively may contribute to a heightened the risk of HIV. The trend of HIV-related deaths was stabilize (static) with years of diagnosis and the death cases are less than alive. Stability of annual HIV-related deaths in Baghdad, reflects in part to the effectiveness of the national HIV program for diagnosis through availability of HIV testing and the success of ART programme increasing survival among people living with HIV.

In this study, both sectors of Baghdad show rising in the annual number of the new HIV cases with years of diagnosis with a higher percent in Reasfa sector. This finding may be related to a higher population density in Resafa than in Karkh sector; and over the past few
years, the presence of massage parlors in Baghdad has significantly increased especially in Resafa sector, raising concerns among authorities in Iraq’s capital that sexually transmitted diseases could spread in salons that secretly provide sexual services may participate in the rising of new HIV cases although the health care services regarding HIV screening, diagnosis, treatment, and prevention are equally distributed in both sectors; in addition to that both sectors sharing the same socio demographic characteristics, religion, cultural behavior.

Regarding to age and sex, the vast majority of the cases were adult males (≥15 years old). This finding was consistent with UNAIDS / WHO.\cite{3} estimate in 2019, that out of 38 million people living with HIV worldwide, 36.2 million people (95%) were adult (≥15 years old) and the remainder (5%) were children (<15 years old), and globally, new IV infections among adult females declined by 17% compared with 9% among adult males. While new HIV cases among children (<15 years old) decreased by 41% \cite{3}. Our results are consistent to Iraqi study published previously by El-Ebadi et al \cite{6} who found that the bulk of HIV cases were males (86.2%) and adults (>20 years old) and also consistent to other studies conducted in Oman \cite{13} and Qatar.\cite{14}

Our findings may be related to the fact that the adult males in Baghdad community are more liberal in movement, travel widely outside the country, more illegal sex practice, and get the infection from his wife. Besides that, as Baghdadi community are more conservative society, social stigma restricted mainly to female HIV infected people with risky behaviors from seeking HIV testing and counseling services made them under estimated.

Epidemiology of HIV transmission is known to be changed over time from one region to another and from one country to another within the same region\cite{15}. In the present study, sexual behavior mainly through heterosexuals, represents the main route of HIV transmission. Previously, from 1986-2005, El-Ebadi et al, found that 84.5% of new HIV Iraqi cases were transmitted by parenteral route mainly through blood donation, while sexual practice is responsible for 11.3% of the cases.\cite{6}

Our finding is consistent to many studies conducted in different Arabian countries of Middle East region where heterosexuals was the most common mode of HIV transmission as in Kuwait, Qatar, UAE, Bahrain, Oman \cite{13}, Syria, Jordan, Egypt, \cite{4} and Palestine \cite{17} and inconsistent to that reported in Iran and Libya , where the main route of transmission was injecting drug use \cite{4}. Our finding is related to the fact that sexual behavior are associated with decreasing religious values, rapid socio-cultural changes, increasing international travel, unemployment , inter-personal factors, increasing costs of marriage. All these factors collectively may participate in increasing the possibility of sexual practices to become the main route of HIV transmission\cite{17}.

During 1986 and 2005, blood donation and perinatal routes were shown to be responsible for 84.6% and 4% of HIV infections in Iraq, respectively \cite{6}; while in our study, blood transfusion is responsible for 4.3% and perinatal for 0.35% of all new HIV cases, this may reflect the effectiveness of blood screening program for blood-borne viral infections since 2010 and indicate that prevention of perinatal transmission of HIV through ante-natal care program had been more effective. In the present study, only one case (0.35%) reported illegal drug injection. This finding doesn’t reflect the actual number because drug addict phenomenon is prohibited in our country and considered as social stigma. However, this leads to increase the percentage of none specify to 13.2% of the cases as unknown cause of HIV transmission.

**Limitations**

Overall, these study findings are important. However, as in all studies, several limitations warrant mentioning. The cumulative annual number of new HIV cases doesn’t reflect the actual number because there are many new HIV cases not registered in the HIV/AIDS program center and are treated out the governmental health settings whether out-side the country or in private clinics. The second limitation is the information biases resulting from incorrect information given to the health authorities especially points regarding routes of sexual transmission especially homosexuals, illegal drug injection, and others) because they are more sensitive in a conservative country like Iraq, so many cases may report none specify (unknown cause) for HIV transmission.
Conclusion

Although there was gradual increasing in the trend of the annual number of HIV cases with time, Baghdad City remains within the area of low HIV prevalence (<0.1%), and there is a potential risk for exposure to an outbreak or epidemic/endemic if no implementation of proper and effective national strategy plans on HIV/AIDS in place. Adult males have a potential risk for acquiring HIV infection. Sexual behavior mainly through heterosexuals was the most common route of HIV transmission.

Acknowledgement: The authors thank with great appreciation to the Iraqi MOH/Department of Public Health/ HIV Medical Center/Baghdad. Also to the College of the Medicine/University of Anbar and Professor Faris Al-lami consultant of Public Health/ Baghdad College of Medicine for their kind help and cooperation to complete this article.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

Molecular Detection of nan, tly and dsA gene of Propionibacterium acnes Isolated from acne vulgaris in Babylon Province

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Abstract
Propionibacterium acnes virulence factors can be divided into chromosomal, plasmid and bacteriophages encoded virulence factors. Many of the Propionibacterium acnes virulence factors, are camp5, gehA, tly, sialidases, neuraminidases, endoglycoceramidases, lipases, and hemolysins genes are clustered in certain areas of the chromosome. A total of 200 clinical acne samples were collected during this study which obtained from patient Suffering from acne vulgaris who to the out-patient clinics and delivery dermatology unite of acne, in hospitals of Babylon Province: AL-Hashmeia general Hospital and Marjan hospital, during the period from April to November 2019. Out of 200 specimens 14 (7.7%) were detected by culture and vitek2 compact9 (64.3%) of them were confirmed by PCR using target gene of Propionibacterium acnes. PCR was conducted to determine the some virulence genes of the isolates by using primers nan, tly and dsA gene. The PCR amplification products were visualized by electrophoresis on 1% agarose gels for 35min at 70v. The sizes of the amplicons were determined by comparison to the 200 bp allelic ladder. Among isolates studies it was found that nan gene present in 6/9(66.7%). isolates, dsA gene it was found in only 3isolates (33.4%) while tly gene it was found in all isolates(100%).

Keywords: Propionibacterium, camp5, gehA, tly, PCR, Isolate.

Introduction
Propionibacterium acnes is a nonspore- forming, gram- positive, anaerobic, pleomorphic rod whose end products of fermentation include propionic acid (1). P.acnes is considered an opportunistic pathogen, causing a range of infections as well as being associated with a number of inflammatory conditions. Its primarily recognized for its role in acne vulgaris where it is thought to contribute to the inflammatory phase of the condition (2).

Propionibacterium acnes, which are a normal inhabitant of the skin, produce fatty acids that inhibit the growth of fungi on the skin. (3). However, when it becomes trapped inside the hair follicle, it may grow and cause inflammation and acne infection (4).

The virulence genes involved in the pathogenesis of acne are camp5, gehA, tly, sialidases, neuraminidases, endoglycoceramidases, lipases, and hemolysins . The lipoglycan-based cell envelope and their extracellular secreted lipase, particularly triacylglycerol lipase, encoded by the gehA gene assists in the adherence and the colonization of the bacterium to the sebaceous follicle. The other product which aids in the acne process by destroying the host tissue includes porphyrins, hyaluronate lyase, endoglycoceramidase, sialidases/ neuramidase, cardiolipin synthetase, and calcineurin like phosphoesterase (5).

Materials and Methods

Samples Collection:
The study involved (200) patients were subjected for sampling which include both skin sites (comedon and pustule) for the sampling were forehead, cheek, forearm, axilla, sole from both sexes and the age of patients ranged from 13 to 30 years. These patients
were diagnosed by dermatology physician, according to the signs and symptoms, in addition to having risk factors that were determined by the information about patients. In this study, patients with recent usage of local antibiotic treatment and usage cosmetic material were excluded from sampling.

**Culturing of Samples:**

Spread a 10 μl (loop full) from the inoculated and incubate thioglygoloy agar and blood agar plates after adding 0.01 ml from Tween-80 and incubate at 37°C at 4-7 days and read plates of *Propionibacterium acnes* suspect colonies thioglygoloy agar and blood agar onto non-selective media, (nutrient agar) plates for morphology and biochemical confirmation of *Propionibacterium acnes*. After culturing of sample used vitek 2 compact system to detection of *Propionibacterium acnes*.

**DNA Extraction for Gram POSITIVE Bacteria**

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Gene aid, UK).

**Confirmed Detection Propionibacterium acnes by PCR using Specific Primer**

The one aim of this study was to develop a rapid molecular diagnostic test to identify and purity of *P. acnes* isolates based on the specific primer (par gene). The primer flanking portion length 1202bp were selected.

**Molecular Detection of Salmonella Typhimurium and Salmonella Enteritidis Group using Multiplex PCR**

PCR mixture was prepared by adding 25 μl of Green master mix (2x) promaga, 8 μl template DNA, 3 μl from forward primer and 3 μl from each four revers primer, final volume was completed to 50 μl by adding nucleuse free water.

**Table 1: The primer sequences and PCR conditions of Propionibacterium acnes.**

<table>
<thead>
<tr>
<th>gene</th>
<th>Primer sequence (5’-3’)</th>
<th>BP</th>
<th>PCR condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>par gene</td>
<td>F-AGCTCGGTGGGTTCTCTCATC-3’</td>
<td>1201</td>
<td>94°C 3min</td>
<td>(Naghdi1 and Ghan (2014)</td>
</tr>
<tr>
<td></td>
<td>R-GCTTCCTCATACCACTGGTCA TC-3</td>
<td></td>
<td>94°C 30sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65°C 45min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 1.30min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 10min 1x</td>
<td></td>
</tr>
</tbody>
</table>

**Detection of Some Virulence Gene Markers by PCR**

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in Table (2). The primer includes *tly* gene, *dsA* gene and *nan* gene. Each 25μl of PCR reaction contained 5μl of each upstream and downstream primer, 5 μl of free nuclease water, 2.5μl of DNA extraction and 12.5 μl of master mix. The PCR amplification products were visualized by electrophoresis on 1% agarose ladder (promega, USA).
### Table 2: Virulence factor primers sequences with their amplicon size Base pair (bp) and their condition

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence (5′-3′)</th>
<th>Size (bp)</th>
<th>PCR condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tly</td>
<td>F5-CAGGACGTGATGGCAATGCGA-3′</td>
<td>909</td>
<td>94ºC 10min 1x</td>
<td>In this study procedure designed</td>
</tr>
<tr>
<td></td>
<td>R5-TCGTTCACAAGACCACAGTGC-3′</td>
<td></td>
<td>94ºC 2min 55ºC 1min 40x 72ºC 1min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72ºC 10min 1x</td>
<td></td>
</tr>
<tr>
<td>nan</td>
<td>F-5-CATCGACGACAATGGGACAC</td>
<td>196</td>
<td>94ºC 10min 1x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-5-TCGGAATAGATCGACTGGGC</td>
<td></td>
<td>94ºC 1min 51ºC 1min 35x 72ºC 1min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72ºC 10min 1x</td>
<td></td>
</tr>
<tr>
<td>dsa</td>
<td>F-ACCATCAACCATCACCAGACT</td>
<td>325</td>
<td>94ºC 10min 1x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-TTCGGATGAGAAGAGCTGCT</td>
<td></td>
<td>94ºC 1min 51ºC 1min 35x 72ºC 1min</td>
<td></td>
</tr>
</tbody>
</table>

### Results and Discussion

The one aim of this study was to develop a rapid molecular diagnostic test to identify and purity of *P. acnes* isolates based on the specific primer (par gene). The primer flanking portion length 1202bp were selected. The result was 9 (64.3%) isolation from *P. acnes* as the following Figure (1).

![Image](image.png)

Figure (1): 1% Agarose gel electrophoresis at 70 volt for 50 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide. L50:1500bp ladder; lane (1-9) were positive for this gene, the size of product is 1202bp.
Propionibacterium acnes on the other hand were considered as normal flora in the skin, however their role in acne is still vague. So this study may concern on this bacteria because there is no molecular studies performed on Propionibacterium acnes, also there is no molecular research in Iraq which covered the importance and pathogenesis of this through its ability to produce and detection certain virulence factor.

N-acetylurnamendas gene (nan gene) was investigated by PCR technique using specific primers for this gene. The result of this experiment indicate for positive amplification as shown in figure (2). Not all samples were gave positive results for nan gene. the size of this genes of product is 196 bp. It was found that nan marker was observed in 6/9 isolates of Propionibacterium acnes (66.7%).

![Figure (2): Agarose gel electrophoresis at 82 volt for 30 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide L100- 3000 bP ladder(abm); lane [1, 2, 3, 5, 6 and 8] were positive for this genes nan gene the size of product is 196bp.](image)

The nanA gene was found to be conserved and sialidase activity was found in P.acnes isolated over a period of 50 years from various geographical locations. The analyzed the sialidase activity of the NanA protein of P.acnes and cloned the sialidase gene nanA. Sialidase is encoded as a precursor protein of 722 amino acids with a 26 amino acid signal peptide. The mature sialidase has a calculated molecular mass of 81 kDa and contains the carbohydrate binding module 32. Sialidase activity does not require the CBM32 domain. The NanA protein is secreted by P.acnes as a dimer.

Molecular studies of dsA gene were done for all Propionibacterium acnes isolates by using specific PCR markers. But in present study not all samples were gave positive results for dsA gene. 3 (33.4%) samples were positive for the dsA genes the size of product is 325bp as shown in figure(3).
Figure (3): Agarose gel electrophoresis at 82 volt for 30 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide L50 - 1500 bP ladder (abm); lane [1, 6, 7] dermatan sulphate-binding adhesins (dsA) gene the size of product is 325bp.

This result was agreement with (8) who detected same strains of Propionibacterium acnes identified two dermatan-sulphate-binding proteins (DsA1& DsA2) with putative phase/antigenic variation signatures and the expression of these proteins by type IA organisms contributes to their role in the pathophysiology of acne and helps explain the recurrent nature of the disease.

Potential Effect of the DsA protein in Propionibacterium acnes are Colonization, adhesion, Fibrinogen-binding and inflammation (4)

The notable virulence gene involved in the pathogenesis of acne was tly gene responsible of damage the blood cells. So, this gene was very important virulence factor for bacteria associated with acne vulgaris. The molecular detection of tly gene was done by using specific primer. It was found that tly gene detected 9 (100%) isolates of Propionibacterium acnes isolates with long length 909 bp as shown in figure (4).

Figure (4): Agarose gel electrophoresis at 82 volt for 30 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide L50 - 1500 bP ladder(abm); lane all P.acnes isolates were positive for this genes Tly gene the size of product is 909bp.
The result correlated with (9) who was showed a basically clonal population structure correlated with allelic variation in the virulence gene tly.

The result correlated with (10) who reporters the 98% identity sequenced of the tly gene from a further 19 P.acnes strains isolated from different sources and selected to represent types I and II. he showed that the differences between type I and II were based on type-specific polymorphisms in tly gene

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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Effect of Cinnamon Gargel on Healing of Aphthous

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Abstract

This study aimed to show the impact of cinnamon mouth gargle on Aphtous ulcer and there role on recovery process of this ulcer.

This take a look at became carried-out on one hundred sufferers affected by Aphtous ulcer that typically visitor clinics.

The sufferers have been divided into groups, Cinnamon dealt with institution: Their wide variety 50 affected person, the aphthus ulcer dealt with Cinnamon gurgle wash 3-instances every day and Placebo dealt with institution. The affected person wide variety became 50 affected person suffered from aphthus ulcer and dealt with placebo 3-instances every day.

Prior to the take a look at, all assigned clinicians from the exclusive medical facilities have been skilled with the aid of using the most important examiner for the same old working way that covered the measurement of ulcers, undertaking the visible analog scale (VAS),and record it.

This study concluded that, the maximum of the sufferers that suffered from Aphtous ulcer typically arise at (30– 40) day of age. Also, the occurrence of Aphtous ulcer decreased in the institution dealt with with Cinnamon also, the ache of Aphthus ulcer decreased with Cinnamon mouth gargle wash.

Keywords: Cinnamon, Gargel, Healing, Aphthous, Ulcer, Oral.

Introduction

With the converting traits and life-style the techniques of oral hygiene status have affected. industrial merchandise of oral health, crafted from artificial products, are favored over herbal re-assets and are being usually used, possibly due to clean availability and on the spot results. But those cleaning and whitening merchandise like toothpaste and mouthwash are being drastically criticized due to their unfavourable results on enamel, gum, mucous membrane.\footnote{1}

for long period many beneficial component that recognized and made from flowers ,may be introduced as protecting material (anti mutagen) to the goods of oral health to keep away from its terrible results on human health. As herbal merchandise, the crucial oils were suggested owning good applications\footnote{2}.

Aphtous stomatitis with recurrent painful aphthous ulcers at the non-keratinized oral mucous membranes.\footnote{3,4}

Chronic recurrent ulcers have 3 type of medical morphology and with distinct time courses. Less than 1 cm minor-type (normally 2–five mm) and then cure without treatment in 4–14 days. These are forming 80–90% from all type of recurrent ulcers\footnote{5}.

Scarring takes place in round 8% of cases of ulcers\footnote{6}. Major ulcers are normally 1–three cm, deep indurated and may remaining from 10 days- six weeks on occasion longer\footnote{7}. They are 10% of recurrent ulcers of oral area. About 64% of these ulcers heal with scarring. Herpetiform aphthous oral ulcers are 1 to 2 mm in

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DOI Number: 10.37506/ijfmt.v15i1.13740
diameter \(^{(8)}\). They account for round five% of recurrent oral aphthous ulcers, are extraordinarily painful, remain for seven to ten days. Herprtiform aphthous appears one hundred ulcers; there will aggregate forming large erosive area, approximately 32% scar forming. \(^{(9)}\).

The efficiency of the oil of cinnamon in DNA molecule was examined and the result then summarized. It changed into located that plant extracts blanketed the DNA from the mutagenic impact of mouth wash and the DNA bands remained intact and have been much like the band of DNA of control. The wells of gel are sparkling due to the presence of crucialoils of remedy because the DNA changed into loaded without delay after remedy to keep away from any impact of purifying answers on DNA structure.

Jayaprakasha et al. \(^{(1)}\) located anti mutagenic pastime of water extract of cinnamon. Also, Hamssa et al. \(^{(10)}\) located black peppers and bell pepper having anti-mutagenic effects. They in addition said that each are succesful of inhibiting carcinogen activation, enhancing the cleansing of carcinogens through scavenging the reactive dealers that harm DNA.

Jayaprakasha et al. \(^{(1)}\) indicated that under-applied and unconventional a part of cinnamon is a great supply of antioxidant and antimutagenic phenolics.

U.S. Department of Agriculture provide that a teaspoon of cinnamon weighing 2.6 g have 6.42 calories of energy; 2.1 g of carbohydrates, 26.1 milligrams (mg) of calcium, 0.21 mg of iron; magnesium: 1.50 six mg, phosphorus: 1.60 six mg, 11.2 mg of potassium and vitamin A: 0.39micrograms. It additionally includes strains of nutrients beta-carotene, alpha-carotene, lycopene, the antioxidants choline, lutein, zeaxanthin and beta-cryptoxanthin, .

Oxidative stress may be decreased by Antioxidants and can decrease the occurrence of cancer, kind 2 diabetes, and plenty of different status. People usually used cinnamon in small amount in food. Therefore, the vitamins it includes will be benefit inside the diet. \(^{(11)}\).

The extract of Cinnamomumzeylanicum includes antioxidant products with the action of hydroxyl radicals and anions from scavenging superoxide. \(^{(12)}\). Inhibiting factor of zeylanicum crucial oil and oxidation factor merchandise formation in mustard oil on the awareness of 0.02% \(^{(11)}\).

Borneolum is acrystal steam distilled product of Cinnamomum camphora \(^{(13)}\). It is for external use in ulceration and sore mouth \(^{(14)}\) and confirmed anti-inflammatory and antioxidant molecular protecting results through reducing inflammatory factor iNOS expression, and NO, release, in addition to NF-κB translocation and associated apoptosis in an ischemic/reperfusion neuron model \(^{(15)}\). Gallachinensis in TCM concept astringes, promotes wound recuperation and is used for ulcers and edema \(^{(16)}\).

This study aimed to examine the impact of cenimon mouth gargle on Aphthous ulcer and there results on recuperation process of this ulcer.

**Patient and methods**

1-Patients:

Patients came to privet clinic how an aphthus ulcers have.

2-Cinnamoon gargle preparation:

The cinnamon spice had been used as a powder mixed with water in appropriate ratio as in hot drink of cinnamon and use as mouthwash treatments.

3-Grouping and statistical design:

Both test and placebo gargle wash (1:1 allocation ratio) had been randomized the usage of a computer-primarily based totally random variety generator and allotted to the centers.

4-The patients had been categorized into:

1-Cinnamon handled organization: Their variety 50, the aphthus ulcer handled with Cinnamon gargle wash 3-instances daily.

2-Placebo handled organization: The affected person variety changed into 50 affected person suffered from aphthus ulcer and handled with placebo 3-instances daily.

5- Study Intervention

The ulcer length and ache level had been examine,
measured and documented on the clinics on 1st, 3, and five day via way of means of assigned unbiased scientific investigators. In addition to pulse, body temperature and blood pressure, had been taken in all visits. When the ulcer heal in the five-day examine period, the cinnamon and/or placebo agent changed into gathered. measured the ulcers dimention, carrying out the visible analog scale (VAS).

6. Clinical notes:

These parkling ulcers, as defined as advanced inside seventy two hrs of onset and actually seen then documented. The assessment of the floor place of the ulcer ,changed appear in cm and mm via way of means of a dental probe (Shanghai,Dental Instrument Factory- China).ulcer diameter usually measured and documented. Pain depth changed into measured the usage of a VAS, in which the amountof ache ranged 0 (no ache) to 10 (insufferable ache). Pain changed into assessed via way of means of annoying the ulcer with the periodontal probe. The values had been gathered via way of means of the assigned investigators.

7-Statistical evaluation:

Statistical evaluation changed into made the usage of SPSSPC + Computer program - Version 25, via the usage of t-check for evaluating among the 2 businesses (Cinnamon handled organization and Placebo handled organization) in ulcer sized development. Also, Chi²-check for evaluating the% of development of in Ulcer length and ulcer ache some of the businesses below the examine.

Results

The baseline demography data of the patients.

Our results observed in Table (1) on the characters of the patients cleared that, the age, of the patients that suffered from Aphtous ulcer not differ significantly among the patients under the studied groups (P > 0.05), the experimental group its age level reached to (32.34 year) and in placebo treated group it reached to (33.17 year).

The results cleared that, the incidences of Aphtous ulcer in experimental group 11 (22 %) lower than its incidences in the placebo treated group in male patients 16 (32 %), while, in female patients in experimental group its incidences reached to 45 (90 %) and in placebo treated group it reached to 35 (70 %).

Also the duration of the previous ulcer not differ significantly among experimental and placebo treated groups as its duration reached to 12 and 11.42 day for Aphtous ulcer in experimental and placebo treated group.

The results cleared that, the experimental group that treated with Cinnamon gurgle wash its average ulcer size reached to 4.51 mm² while, in placebo treated group its size reached to 5.14 mm².

The results observed in Table (1) cleared that the ulcer pain (VAS) in Cinnamon treated group is lower than its level in the placebo treated group.

| Table (1): distribution data of the patients. |
|-----------------|-----------------|--------|
|                  | cinnamon group (n =50) | Placebo group (n =50) | P   |
| (Age per year) (mean ± SD)* | 32.34 ± 10.12 | 33.17 ± 11.9 | 0.77 |
| Gender**                |                  |                    |
| Male,                   | 11 (22%)         | 16 (32%)          | 0.016 |
| Female,                 | 45 (90 %□)       | 35 (70%)          |      |
| Duration of previous ulcer (day)* | 12 ± 3.96 | 11.42 ± 4.16 | 0.95 |
Our results on the improvement of the ulcer size among patients treated with Cinnamon gurgling wash and placebo wash:

The results observed in Table (2), cleared that, the experimental group that treated with Cinnamon mouth gurgling have lower size of ulcer than the placebo treated group as its size reached to 3.2 mm² in cinnamon treated group, and reached to 4.3 mm² in placebo treated group at 3 days of treatment, while, at 5-days post-treatment its size reached to 2.1 for Cinnamon treated group and 3.7 mm² in placebo mouth wash treated group, respectively.

Also, the improvement % at 3 and 5-days post treatment improved in Cinnamon using group than the placebo group for improvement % in Cinnamon at day 3 was 14 (28 %) and in placebo treated group it was 9 (18 %). While, at 5-day post-treatment the improvement % reached to 35 (70 %) in Cinnamon treated group, while, in placebo treated group it reached to 28 (56 %).

The effective indices for significant improvement cleared that the Cinnamoen is more efficient in treatment of Aphthus ulcer than the placebo treatment at 3 and 5 days of experiment. While, the non-significant improvement showed a higher results in placebo than the Cinnamon treatment of Aphthus ulcers.

### Table (1): distribution data of the patients.

<table>
<thead>
<tr>
<th>Size of ulcer (mm²) (mean ± SD)*</th>
<th>4.51 ± 3.12</th>
<th>5.14 ± 4.15</th>
<th>0.18</th>
<th>Chi² = 0.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain of ulcer (VAS)***</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VAS: visual analog scale.

**= t-test for comparison between the gender groups.
Table (2): NO. of person in cinnamon and placebo groups on day 3 and day 5 depending on diameter of ulcer.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>3-Day</th>
<th></th>
<th>5-Day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental group (%)</td>
<td>Placebo group (%)</td>
<td>P</td>
<td>Experimental group (%)</td>
</tr>
<tr>
<td>Number</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Size (mean ± SD)</td>
<td>3.2 ± 2.1</td>
<td>4.3 ± 1.52</td>
<td>0.01*</td>
<td>2.1 ± 1.50</td>
</tr>
<tr>
<td>Significant improvement</td>
<td>14 (28%)</td>
<td>9 (18%)</td>
<td>0.03***</td>
<td>35 (70%)</td>
</tr>
<tr>
<td>EI size = 100%</td>
<td>5 (10%)</td>
<td>3 (6%)</td>
<td>18 (36%)</td>
<td>24 (21.1)</td>
</tr>
<tr>
<td>EI size = 70–100%</td>
<td>10 (20%)</td>
<td>6 (12%)</td>
<td>20 (40%)</td>
<td>33 (28.9)</td>
</tr>
<tr>
<td>Nonsignificant improvement</td>
<td>35 (70%)</td>
<td>45 (90%)</td>
<td>12 (24%)</td>
<td>57 (50.0)</td>
</tr>
<tr>
<td>EI size = 30–70%</td>
<td>20 (40%)</td>
<td>19 (38%)</td>
<td>8 (16%)</td>
<td>21 (18.4)</td>
</tr>
<tr>
<td>EI size = 0–30%</td>
<td>30 (60%)</td>
<td>26 (52%)</td>
<td>11 (22%)</td>
<td>36 (31.6)</td>
</tr>
</tbody>
</table>

*and **: P values the differences of ulcer size on groups in 3 and 5 days. between groups on days 3 and 5.

***and ****: P values indicate the comparisons of significant improvement and non-significant improvement at 3 and 5 days.

EI = Effective indices

Our results on the improvement of the ulcer pain among patients treated with Cinnamon gurgle wash and placebo wash:

The VAS was used to measure the level of pain for both groups. The results observed in Table (3), cleared that, the improvement % of pain at 3 and 5 days post treatment improved in Cinnamon group than the placebo treated group as the improvement % in Cinnamon at day 3 was 14 (28%) and in placebo treated group it was 9 (18%). While, at 5-day post-treatment the pain improvement % reached to 36 (72.00 %) in Cinnamon treated group, while, in placebo treated group it reached to 29 (58.0 %).

The effective pain indices for significant improvement cleared that the Cinnamoen is more efficient in treatment of Aphthus ulcer than the placebo in reducing the pain at 3 and 5 days of experiment.

While, the non-significant improvement showed a higher results in placebo than the Cinnamon treatment of Aphthus ulcers.
Table (3): NO. of patients in cinnamon and experimental groups on day 3 and day 5 according to severity of pain.

<table>
<thead>
<tr>
<th></th>
<th>3-Day</th>
<th>5-Day</th>
<th>P</th>
<th>3-Day</th>
<th>5-Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cinnamon group (%)</td>
<td>Placebo group (%)</td>
<td></td>
<td>Cinnamon group (%)</td>
<td>Placebo group (%)</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>50</td>
<td>50</td>
<td></td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Significant improvement</td>
<td>14 (28 %)</td>
<td>9 (18 %)</td>
<td>&gt;0.05*</td>
<td>36 (72 %)</td>
<td>29 (58 %)</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>EI pain = 100%</td>
<td>8 (16 %)</td>
<td>3 (6 %)</td>
<td>24 (48 %)</td>
<td>22 (44 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI pain = 70–100%</td>
<td>8 (16 %)</td>
<td>6 (12 %)</td>
<td>13 (26 %)</td>
<td>7 (14 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsignificant improvement</td>
<td>42 (84 %)</td>
<td>48 (96 %)</td>
<td>19 (38 %)</td>
<td>27 (54 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI pain = 30–70%</td>
<td>17 (34 %)</td>
<td>21 (42 %)</td>
<td>12 (24 %)</td>
<td>16 (32 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EI pain = 0–30%</td>
<td>25 (50 %)</td>
<td>27 (54 %)</td>
<td>7 (14 %)</td>
<td>11 (22 %)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*and **: P values represent the comparisons of ulcer pain between groups on days 3 and 5, respectively.

***and ****: P values represent the comparisons of significant improvement and nonsignificant improvement on days 3 and 5, respectively.

EI = Effective indices

**Discussion**

25% is the percentage of the prevalence of Recurrent aphthous stomatitis (RAS) (17), which occur on oral mucosa which is non keratinized. There are 3 main type of oral ulcers major, minor also, herpetiform (18), 80–85% of RAS, with 3–10mm ulcers usually painful and appears on groups greater than 5 lesions heal between 10 days to two week (18),there are no specific treatments for RAS depending on the etiology, but we must work to decrease the pain and the duration of ulcers by prevent the secondary infection and suppress the local immune factors (17).

Our results on this study: cleared that, the most of the patients that suffered from Aphthous ulcer commonly occur at (30 – 40) day of age and in female higher than male.

This results attributed Recurrent Aphthous ulcers (RAS) is common manifestation that related to group of health problems with multiple causes (19). The development of ulcers depending on the local Immune factors, which was supported by the histological infiltration of plasma cells, lymphocytes, and neutrophils of oral mucosa (20). There are many types of cytokines played a role on the pathogenicity of ulcers (21), in RAS lesions the IL-10 concentration decreased, while IL-2, IFN-γ, and TNF-α were elevated (22,23).

The results cleared that, the incidences of Aphabetous ulcer reduced in the group treated with Cinnamon.

The present of ulcers in recurrent aphthus stomatitis
patients were usually exposed to huge number of microorganisms, then lead to inflammatory reaction. The healing of ulcers depends on the protection against microbes. Although the antimicrobial activity of Cinnamon on inflammatory disease was denied by Li et al. because there is no reported effects on *E. coli* growth (24).

This results agreed with those of (1,25) where they reported that, cinnamon water extract have anti-mutagenic activity and it preserve the health condition of the cells and help in regeneration of the cells and treat the ulcer with reduction of its size.

Also the pain reduced in the group treated with Cinnamone, this results attributed to with the healing of many ulcers, the pain of ulcers will be disappeared. The activity of Cinnamon gurgle wash on pain, explain in this study on day 5, may be due to healing of ulcers, and not belong to the analgesic effect of cinnamon.

This results agreed with those of (18) where they reported that by using some herbs as Cinnamon can treat the unclear etiology of the ulcer, by decrease the pain and the duration of ulcers via prevent the secondary infection and suppress the local immune factors.

The active effect of cinnamon mouth wash in treatment of aphthus ulcer attributed to the Cinnamon contain Cinnamaldehyde (CM) which is the active component of the spice cinnamon (*Cinnamomum zeylanicum*). It had anti-microbial, anti-inflammatory, anti-tumor, anti-oxidant, cholesterol lowering and immunomodulatory properties (26). Cinnamon consider as anti-inflammatory such as in gastric inflammation by decrease the activation of NF-κB (27). Cinnamon effects on regulatory T-cells which lead to decrease allergic encephalomyelitis in vivo (28). Cinnamon consider as anti-rheumatic agent due to their action on reduce the inflammation in arthritis in vivo by decrease the effect of cytokines such as interferon γ (IFNγ) and, IL-2, IL-4, (29). Cinnamon is effective in the treatment of degenerative disease of neurological origins as in AD (30, 31).

Our results concluded that, the most of the patients that suffered from Aphhtous ulcer commonly occur at (30 – 40) day of age. Also, the incidences of Aphhtous ulcer reduced in the group treated with Cinnamon also, the pain of aphthus ulcer reduced with Cinnamon mouth gurgle wash.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


GC-Mass Spectrometry Analysis of Iraqi *Moringa Oleifera* Seeds Extract

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Abstract

*Moringa oleifera* is an important traditional medicinal plant used for the treatment of many health problems. It is widely cultivated across the world but it has not well known or well-studied in Iraq. Therefore, the aim of this study is to identify the bioactive compounds present in acetone:methanol (1:1) seeds extract of Iraqi *Moringa oleifera* by using GC-Mass Spectrophotometry.

The results of this analysis revealed 41 compounds which include, terpenes (cis-beta-Ocimene 0.97%, Cedrene 0.20%, Cedrenol 1.40%, Verrucarol 0.29%, Dehydroxy-isocalamendiol 0.09%, beta-Myrcene 0.14%, 3-Thujene 0.06%, Ocimene 0.72%, Sabinene 0.37%, Aromadendrene 1.54% ), phenols (0.34%, Eugenol 0.49%, cis-Isoeugenol 1.21%, Methoxyeugenol 0.26%, Ferrocene 0.57%, Phenol, 2-methoxy-4-(1-propenyl)-, (Z) 0.34, Oxabicyclo[3.3.0]octan-2-one -7-neopentylidene) 0.61% ), fatty acid methyl esters (n-Tridecanoic acid 0.15%, Undecanoic acid 0.09%, Pentadecanoic acid 34.43%, 4-Hexadienal 1.52%, Dodecanoic acid 11.02%, cis-Vaccenic acid 21.39%, Hexadecenoic acid 0.15%, Eicosanoic acid 0.03%), acid (myristicin, 7.8%), Phenyl propanoid (Asarone, 4.99%), Antioxidant (tert-Butyl-4-hydroxyanisole, 1.38%), Drug (Berbine, 2,3,9,10-tetramethoxy, 0.73%), Drug (Thiazolo, 0.63%), aldehyde (Trimethylsilyl vanillin, 0.61%), hormone (Octenoic acid, 0.46%), Amine (ethyl ester, 0.42%), Carboxylic acid (Dimethylandrost5-en-3-one, 0.28%), Terpenoid (Lavandulol, 0.19%), Heterocyclic compound (Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl, 0.18%), Volatile oil (Butanoic acid, 0.16%), Fatty alcohol (1-Heptatriacontanol, 0.12%), Hydroxy carbon-alkene-(Carene, 0.11%).

It can be concluded from this study that the seeds are considered a rich source of bioactive compounds.

**Keywords:** GC-MS analysis, Bioactive compounds, Methanol:Acetone Extract, Iraqi *Moringa oleifera*.

Introduction

Medical plants are commonly used as a source of medicinal products or against the toxicity of xenobiotics. World Health Organization reports that 4 billion people (80 percent of the world’s population) use herbal medicinal goods for most of the primary health care.

*Moringa oleifera* is among 13 species of Moringa trees that grow in different countries around the world.

Seeds of *M. oleifera* tree are useful for medicinal, practical food preparations, water purification, and biodiesel production. *M. oleifera* seeds are a good source of proteins, lipids, vitamins, minerals, micronutrients, and bioactive phytochemicals. The aqueous seeds extract of *M. oleifera* inhibit bacterial and fungal growth. The methanol: acetone seed extract of Iraqi *M. oleifera* is found to reduce the toxicity of nanoparticles of lead in the rat (Unpublished results).

This study aims to estimate the compounds found in methanol: acetone seeds extract of Iraq *M. oleifera* by using GC mass spectroscopy.
Material and Methods

Plant collection

The ripe dried pods of *M. oleifera* were collected from the trees planted in National Iraqi herbarium (in Baghdad), Iraq, and was identified by a taxonomist in the Desert center, University of Anbar, Iraq.

Preparation of samples

The seeds were removed from dried pods, dehusked, kept at shade ambient temperature (45°C) to remove any possible moisture. The seeds were later milled to obtain seed powder using a blender and stored it in the refrigerator in airtight containers for analysis.

Preparation of extract

The dried powdered seeds (300gms) were extracted using Soxhlet and thimble with 1000 ml of solvent (methanol: Acetone 1:1) for 48 hours. The extract was then evaporated with a rotary evaporator. The dried extract was stored at 4°C in a sterile container.

GC-MS analysis

The chromatograph used was Shimadzu GC-2010 plus-Japan in the Iraqi ministry of sciences and technology; Column: ZB-5MS Capillary Column (30 m x 0.25 mm, I.D. 0.25 μm), Carrier Gas: UHP Helium, Injection Temperature: 280.00°C, Detector Temperature: 280.00 °C, Injection Mode: Split, Flow Control Mode: Pressure, Injector Pressure: 100.0 kPa, Total Flow: 47.3 μl/min, Column Flow: 1.43 μl/min, Linear Velocity: 44.1 cm/min, Injection Volume: 1 μL, Run Time: 35 minutes. A freshly prepared sample (10mg/ml) was used.

Results and Discussion

GC-MS is an important device used for the analysis of bioactive chemical constituents according to their mass to charge ratio.

Forty one bioactive compounds were identified in seeds extract (methanol:acetone 1:1) of Iraqi *M. oleifera* by GC-MS analysis and detailed in Figure 1., the compounds included terpenes (cis-beta-Ocimene 0.97%, Cedrene 0.20%, Cedrenol 1.40%, Verrucarol 0.29%, Dehydroxy-isocalamendiol 0.09%, beta-Myrcene 0.14%, 3-Thujene 0.06%, Ocimene 0.72%, Sabinene 0.37% Aromadendrene 1.54%) , phenols (0.34%, Eugenol 0.49%, cis-Isoeugenol 1.21%, Methoxyeugenol 0.26%, Ferrocene 0.57%, Phenol, 2-methoxy-4-(1-propenyl)-, (Z) 0.34 , Oxabicyclo[3.3.0]octan-2-one -7-neopentyldiene) 0.61% ), fatty acid methyl esters ( n-Tridecanoic acid 0.15%, Undecanoic acid 0.09%, Pentadecanoic acid 34.43%, 4-Hexadienal 1.52%, Dodecanoic acid 11.02%, cis-Vaccenic acid 21.39%, Hexadecenoic acid 0.15%, Eicosanoic acid 0.03%), acid (myristicin, 7.8%), Phenyl propanoid (Asarone, 4.99%), Antioxidant (-tert-Butyl-4-hydroxyanisole, 1.38%), Drug (Berbine, 2,3,9,10-tetramethoxy, 0.73%), Drug (Thiazolo, 0.63%), aldehyde (Trimethylsilyl vanillin, 0.61%), hormone (Octenoic acid, 0.46%), Amine (ethyl ester, 0.42%), Carboxylic acid (Dimethyleneostrost-5-en-3-one, 0.28%), Terpenoid (Lavandulol, 0.19%), Heterocyclic compound (Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl, 0.18%), Volatile oil (Butanoic acid, 0.16%), Fatty alcohol (1-Heptatriacontanol, 0.12%), Hydroxy carbon-alkene-(Carene, 0.11%).

The following examples revealed the biological activity of each of the above compounds. Myristicin was found to act as prophylactic effects against colitis in mice, the myristicin treatment before the induction of colitis significantly changed the colonic oxidative stress by elevating the antioxidant enzymatic activities, and reducing the lipid peroxidation (11).

Asarone may be developed to a therapeutic agent to manage cognitive weakening related to conditions such as Alzheimer (12) and increase of chemosensitivity by preventing tumor glycolysis in gastric cancer (13).

Methoxyeugenol has anticancer activity and antimicrobial activity (14). Pentadecanoic acid, suppresses the stemness of mcf-7/sc human breast cancer stem-like cells through JAK2/STAT3 signaling (15). Dodecanoic acid, may elicit apoptosis in certain cancer cells (16) and exerted antiproliferative activity in diverse types of tumor cells (17). When fed vaccenic acid over 16 weeks, rats exhibited lowered total cholesterol, lowered LDL cholesterol, and lower triglycerides levels (18).

The crude seeds extract of *M. oleifera* was also found to have protective effects against the toxicity of lead nanoparticles in rats (Unpublished results), oxidative DNA damage (19), and against bacterial growth (20).
Conclusions

The result of a GC-mass analysis of Iraqi *M. oleifera* was valuable. The number and quality of the compounds manufactured and stored in the seeds of *M. oleifera* reveal the nutritional and/or medicinal importance of these phytochemicals. The compounds, pentadecanoic acid, Cis-Vaccenic acid, dodecanoic acid, and myristicin constitute about 74.64% of the total contents of extraction.

Figure (1): TIC of the GC-MSS of methanol: acetone (1:1) seeds extract of Iraqi *M. oleifera*

A- Myristicin

B- Asarone

C- Methoxyeugenol
Figure 2: Some important compounds in methanol: acetone (1:1) seeds extract of Iraqi M. oleifera

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Correlation Between Serum Ferritin And Liver Function in Thalassemia Patients

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Abstract

Thalassemia, is one of the most important hemolytic genetic, diseases that break down red blood cells, Children with thalassemia cannot produce enough hemoglobin because; the bone marrow cannot produce enough red blood cells to meet their needs and the red blood cells produced, by them are almost devoid of hemoglobin, and the disease is divided into alpha, and Beta thalassemia.

The current study was designed with the aim of investigating the relationship between serum ferritin and hepatic enzymes in thalassemia patients. Iron is stored primarily in the form of ferritin in liver cells. The study included 60 patients with thalassemia in; Dhi Qar governorate, distributed between the two gender during the research period, their ages ranged between (2-26) years old, blood samples were taken in order to testing of, hemoglobin, ferritin, and liver enzymes (ATP, GOT, GPT), and the same, tests were performed on healthy people 40 person; as they are a control group.

The results recorded; a high percentage of thalassemia incidence in males and a decrease in females. The study also showed; that the incidence of thalassemia reached its peak in the age. group (2-6) years and decreased with age, as it reached its lowest levels in the age, group (22-26) years.

The study revealed; a significant decrease (0.05 > P) in hemoglobin concentration in thalassemia patients compared with the control group, and the results recorded a significant; increase (0.05 P <) in the concentration of ferritin in thalassemia patients compared with the control group.

The current study; also showed a significant increase (P <0.05) in GPT enzyme concentration in thalassemia patients compared with the control group. There was a significant increase (P <0.05) of GOT enzyme concentration in patients compared to the control group.

The results indicated; that there was a significant increase (P <0.05) in the concentration of the ALP enzyme in the patients compared with the control group.

Keywords: thalassemia, ferritin, liver

Introduction

Thalassemia: It is one of the most important hereditary hemolytic diseases and the most prevalent in the world, Thalassemia is a Greek word in origin that means anemia in the Mediterranean region. The disease is widely known in this region and is also known as Mediterranean anemia. In the United States of America, it was known as Cooley’s anemia, due to the disease’s discoverer who discovered it in 1925 (1)
Thalassemia is often diagnosed in the first six months of a newborn’s life, and it may be fatal if the patient does not receive appropriate treatment. Children with thalassemia need a blood transfusion every 3-4 weeks, depending on the patient’s age and the degree of hemoglobin deficiency in the blood in order to be able to survive.

Thalassemia major disease does not affect the fetus in the uterus, because fetuses have a special type of hemoglobin called fetal hemoglobin and differs from adult hemoglobin, and when a fetus is born most of the hemoglobin in its body remains the hemoglobin of fetuses in the first six months of life, but the problem of thalassemia lies in the inability of children to generate sufficient amounts of hemoglobin, so thalassemia, major symptoms begin to appear in children in the first year of life.

The lifespan of red blood cells in thalassemia patients is very short, so these pellets are gradually eaten, causing severe anemia, and so patients undergo periodic blood transfusions to keep the red blood cells close to the required level, which improves, the patient’s condition and ensures the transfer of oxygen to the tissues and the normal growth of the body. Anemia worsens and the child’s growth stops. As a result, the shape of the child’s forehead and bones changes.

Types of Thalassemia:

1- Alpha-thalassemia:

It is uncommon and has been found periodically in different parts of the world. There are four alpha globin genes, so the disease symptoms appear according to the number of defective genes:

- Silent alpha thalassemia:
  It occurs if the mutation affects only one gene, then the person does not have any health problem.

- Alpha thalassemia trait carrier:
  If the mutation affects only two of the genes and it does not accompany any health problem, but children may be born to him with health problems in hemoglobin if his wife is a carrier of the same trait.

1- Beta-thalassemia:

It is the most common genetic disorder worldwide, there are two beta globin genes, so the symptoms of the disease appear according to the number of defective genes:

- Thalassemia minor (carriers of the disease):
  When there is a defect in one of the beta globin genes, this condition is called a carrier of the disease, and does not suffer from any health problem, except from a slight anemia that does not require a blood transfusion, he is able to transmit the disease to his children.

- Medium thalassemia:
  It occurs when there is a defect (mutation) in both of the beta globin genes, resulting in a moderate decrease in the amount of beta globin produced in the body, leading to a moderately severe decrease in the hemoglobin level in the blood, and the patient does not need a periodic transfusion.

- Thalassemia major:
  It occurs when there is a defect (mutation) in both of the beta globin genes, but the type of damage in the beta gene this time is more severe. This results in a severe decrease in the proportion of beta-globin, thus reducing hemoglobin as a result of breaking down the abnormal red blood cells before the end of their life span, The patient needs periodic blood transfusions every 3-4 weeks to maintain a high level of Hb in order for the
body, to grow healthy (11).

Aim of Study:

In light of the foregoing, the present study suggested in order to investigate the effect of thalassemia infection on liver function by studying the following criteria:

1- Measuring the levels of liver enzymes represented by GOT, GPT and ALT.

2- Measurement of serum ferritin level (iron-storing protein).

3- Measurement of hemoglobin level in the blood.

4- To identify the largest infection rate among different age groups.

5- Comparing the infection rate between the two gender and, investigating the causes.

Materials and methods:

1-Blood samples:

Blood samples were drawn from thalassemia patients during their visit to the General Al-Habobi Hospital / Genetic Hematology Center in order to receive treatment and care, (60) samples were obtained from these patients distributed between of either gender. during the research period, their ages ranged between (2-20) years, and examinations were performed in hematology and biochemistry laboratories.

For the period from December / 2018 to July / 2019, 5 ml of blood was taken for each patient and a hemoglobin,(Hb) test was performed in the blood laboratory, the blood serum was obtained by placing samples in clean and dry test tubes then placed in a centrifuge At a speed of ;4000 r / min for five minutes and the serum was separated from the thrombus by a micro pipette, the serum was placed in labeled Eppendorf tubes for the examination of ferritin and liver enzymes (GPT, GOT, ALT).The same amount of blood was drawn from healthy people (40) samples and they underwent the same tests for comparison purposes as control samples.

2- Hemoglobin measurement:

Hemoglobin was measured using the Coulter Horiba blood tester of French origin Emerald. company.

3- Liver enzymes measurement:

3-1- Measurement of Aspartate transaminase (AST) GOT:

This enzyme; was measured by using a kit of the type. bio Merieux- French, and the, activity of this enzyme was measured by color methods by using the method of .(Gella F.J. et al., 1985), according to the following reaction equation:

$$\text{Aspartate} + \alpha \text{Ketoglutarate} \leftrightarrow \text{Oxaloacetate} + \text{glutamate}$$

The formed oxaloacetate is measured by color methods through, its interaction with 2,4-. dinitrophenylhydrazine.-.to form the hydrozone derivative. This derivative; is measured through a spectrophotometer .with a wave length of 505nm, the .intensity of absorption will be proportional. to the activity of this enzyme.

3-2- Measurement of Alanine transaminase (ALT) GPT:

The effectiveness, of this enzyme was measured using a bio Merieux- French kit, and GPT (Glutamic pyruvic transaminase) ,was measured by color methods which described .by (Lustig V. et al., 1988) according to the following. reaction:

$$\text{GPT}$$

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \rightarrow \text{Pyruvate} + \text{Glutamate}$$

The liberated pyruvate is. measured by the presence of the compound 2- 4- di-nitrophenyl hydrazine .to form the ,hydrazone ,derivative, and this compound is measured by a spectrophotometer; with a wave length of 505nm. The. intensity of absorbance, will be proportional. to the activity of this enzyme.

3-3- Measurement of Alkaline, phosphatase (ALP):

This enzyme was; measured by several bio Merieux- france kit, and the basis of this method depends on the. activity of this enzyme in the basic medium by using the. method described by Tietz , et al. (12) according to the
following equation:

\[
\text{Alkaline Phosphatase} \\
\text{Phenylphosphate} \xrightarrow{\text{Phosphate}} \text{Phenol} \\
\text{PH} 10
\]

The released phenol is measured by the presence of the 4-aminoantipyren compound, and the potassium, Ferro cyanide. The presence of sodium arsenate is to stop the enzyme activity. Then the solution is measured over a wavelength of 510 nm. The amount of absorption is proportional to the amount of phenol released; and this depends on the activity of the enzyme.

**4- Measuring the level of ferritin:**

The method for quantifying protein storage, ziron (Ferritin) is based on the immunoassay using the method which described by Gomez et al (13). The antibody is used for a specific type of mice and is surrounded by a layer of magnetic atoms, which represent, the solid phase, and another antibody for a type of rat linked to derivatives of Isoluminol. The work steps were carried out according to the instructions of the Italian company DiaSorin, and equipped with the equipment needed to measure the concentration of Ferritin.

**5- Determining the incidence of thalassemia among males and females:**

\[
\text{Class number) Percentage of thalassemia, incidence)%} \\
\text{=} \frac{\text{__________}}{\text{the total number}} \times 100
\]

**6- Divide thalassemia patients into five age groups:**

- The first age group. (2-6) years.
- The second age group. (7-11) years.
- The third age group. (12-16) years.
- The fourth age group. (17-21) years.
- The Fifth age group. (22-26) years.

**7- Statistical analysis:**

The results were analyzed by using the SPSS 17 statistical program to determine the mean, standard deviation, and other variables. The differences in the case of the probability \( p < 0.05 \) were considered statistically significant.

**Results**

**1 - Thalassemia relationship with gender:**

The current study, indicated that there, was a high percentage of thalassemia in males, which was 61.70%, while in females it decreased to 38.30%.
2 - Thalassemia relationship with age:

The current study showed that the incidence of thalassemia reached its peak in the first age group (2-6) years, so the percentage was (37.26%), then the infection rate decreases, as the age progresses until the ratio gradually decreases to the lowest level in the, fifth age group (22-26 years), so it was (3.14%).

![Figure 2: Distribution of age groups in thalassemia patients.](image)

3 - Thalassemia effect on concentration of hemoglobin and Serum ferritin:

The results of the current study showed a significant decrease (P <0.05) in the hemoglobin Hb concentration in Thalassemia patients group (2) with a concentration of (7.76 ± 0.58 g / dL) compared to the control group (1) it was (13.40 ± 0.92) g / dL.

The results recorded, a significant increase (P<0.05) in the concentration of ferritin in thalassemia, patients, group (2), it reached (3841.79 ± 942 μg / L) compared to the control .group (1) as it was (150.69 ± 44.72 /g / L) as shown In Table (1).

<table>
<thead>
<tr>
<th>Studied Parameters groups</th>
<th>Serum ferritin (μg/L)</th>
<th>Hb (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Control group</td>
<td>150.69±44.72 b</td>
<td>13.40±0.92 a</td>
</tr>
<tr>
<td>(2) Thalassemia group</td>
<td>3841.79±942 a</td>
<td>7.76±0.58 b</td>
</tr>
<tr>
<td>LSD</td>
<td>237.89</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The values represent the mean ± the standard deviation.

The letters a, b indicate a significant difference (P <0.05) between the studied groups depending on the value of LSD.
4 - Effect of Thalassemia on liver enzymes levels:

The results of the current study showed a significant increase (P < 0.05) with GPT enzyme concentration in Thalassemia patients (Group 2), it was (29.74 ± 5.40 U/ml) compared to the control group (Group 1) which reached (17.04 ± 2.07 U/ml).

The results also recorded a significant increase (P < 0.05) with a concentration of GOT enzyme in group (2) reached (30.02 ± 7.23 U/ml) compared to control group (1) was (13.87 ± 0.70 U/ml).

The results indicated a significant increase (P < 0.05) with the concentration of ALP enzyme in group (2), it was (166.23 ± 8.45 U/ml) compared to the control group (1) that recorded (81.06 ± 26.79 U/ml).

Table (2): The effect of thalassemia on liver enzyme levels.

<table>
<thead>
<tr>
<th>LSD</th>
<th>(2) Thalassemia group</th>
<th>(1) Control group</th>
<th>Groups Studied Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.45</td>
<td>29.74±5.40 a</td>
<td>17.04±2.07 b</td>
<td>GPT (U/ml)</td>
</tr>
<tr>
<td>1.84</td>
<td>30.02±7.23 a</td>
<td>13.87±0.70 b</td>
<td>GOT (U/ml)</td>
</tr>
<tr>
<td>7.01</td>
<td>166.23±8.45 a</td>
<td>81.06±26.79 b</td>
<td>ALP (U/ml)</td>
</tr>
</tbody>
</table>

- The values represent the mean ± the standard deviation.

- The letters a, b indicate a significant difference (P < 0.05) between the studied groups depending on the value of LSD.

Discussion

1 - Thalassemia relationship with gender:

It is clear from Figure No. 1 that the males are more infected than females. The percentage, of thalassemia in males was 61.70%, while in females it decreased to 38.30%. There is no clear explanation for this, although it is consistent with previous studies (4), but the reason may be attributed to the possibility of a genetic association, with gender, as it may reflect the reality of the areas where the disease is spreading where increased attention to males compared to females, in addition to the reluctance; of some families to visit hospitals when; the disease is present in females.

2 - Thalassemia relationship with age:

The current study showed in Figure (2) that the incidence of thalassemia reached its peak in the first age group (2-6) years, ;so the ratio was (37.26%), and the incidence rate gradually decreased with age, until it reached the lowest level in the fifth, age group (22-26) year, it was (3.14%), and the reason for this may be an increase, in deaths of children with thalassemia, as the age progresses, either due to the cost of treatment, which is offset by the poor physical condition of the patients, the reason may be the delayed of disease diagnosis to advanced stages, when the treatment may be not benefit with it, or the cause may be attributed to the patient’s poor health condition and suffering from severe complications of the disease that may eventually lead to death (3).

3 - Thalassemia effect on concentration of hemoglobin and Serum ferritin:

It was noted through the results of the current study in Table (1) that there was a significant decrease (P < 0.05) in the hemoglobin, concentration in thalassemia patients, as its concentration (7.76 ± 0.58 g / dL) compared to the control group was (13.40 ± 0.92 g
Indian Journal of Forensic Medicine & Toxicology, January-March 2021, Vol. 15, No. 1

(μg/L), and the reason for this may be attributed to the genetic mutation occurring in both genes of beta-globin, resulting in a severe decrease in the ratio of beta-globin, thus a decrease in hemoglobin, as a result of the breakage of abnormal erythrocytes before the end of their shelf life 120 days (11). The Erythropoiesis process is ineffective and manifests itself through the apoptosis of erythrocytes as a result of obstructing the manufacture of heme units, as hemoglobin is mainly composed of two parts, heme and globin protein, so the resulting red blood cells are in small size, small numbers and low hemoglobin, and that high fracture due to the decrease severe hemoglobin concentration, and the results of the current study are consistent with what it reached (14), and this condition occurs when two of the damaged gene carriers of beta thalassemia decide marriage and childbearing, as a result of which the patient will need to periodically blood transfer every 3-4 weeks to maintain a high rate of Hb to allow the body to grow in a healthy way (4).

Because thalassemia is the most common genetic defect in humans, around the world (15), genetic blood disorders are characterized by a low level of hemoglobin (Hb) in red blood cells (RBC) and a very low production of red blood cells (6), and although this disease is characterized by a number of signs and symptoms, severe anemia is the only crucial thing (10).

The results recorded a significant increase (P <0.05) in the concentration of ferritin in thalassemia patients, as it reached (3841.79 ± 942 μg/L) compared to the control group, as it was 150.69 ± 44.72 μg/L, and the reason for this may be periodic blood transfusions every 3-4 weeks to maintain a high rate of Hb to allow the body to grow in a healthy way (4).

Because thalassemia is the most common genetic defect in humans, around the world (15), genetic blood disorders are characterized by a low level of hemoglobin (Hb) in red blood cells (RBC) and a very low production of red blood cells (6), and although this disease is characterized by a number of signs and symptoms, severe anemia is the only crucial thing (10).

The results recorded a significant increase (P <0.05) in the concentration of ferritin in thalassemia patients, as it reached (3841.79 ± 942 μg/L) compared to the control group, as it was 150.69 ± 44.72 μg/L, and the reason for this may be periodic blood transfusions every 3-4 weeks, as multiple blood transfusions lead to increased iron loading, which increases its sedimentation in the body, which in turn leads to an increase in ferritin levels. Thus, iron levels in the blood serum of patients. Thus, iron levels in the blood serum increase as they increase, and repeat blood transfusions (5). Iron is stored primarily in the liver at 70%, and the remainder is stored in the spleen and bone marrow in the form of ferritin in addition to the presence of additional amounts of it in the blood (3).

When conducting blood transfusions to thalassemia patients, the percentage of iron will increase, which leads to the inability of the ferritin to absorb it. Ultimately, free iron is deposited in the blood and tissues (16). This iron burden is usually reflected by increasing serum ferritin levels and controlling it on Long-term correlation with survival, estimating the concentration of ferritin in the blood is an indicator for measuring total iron in the body (15).

4 - Effect of Thalassemia on liver enzymes levels:

Table 2 shows a significant increase (P <0.05) in GPT enzyme concentration in Thalassemia patients, as it was 29.74 ± 5.40 U/ml compared to the control group that reached (U/ml 17.04 ± 2.07). The results also recorded a significant increase (P <0.05) in a concentration of GOT enzyme in Thalassemia patients, as it reached (30.02 ± 7.23 U/ml) compared to the control group that was (U/ml) 13.87 ± 0.70. The results indicated a significant increase (P <0.05) in the ALP enzyme concentration of thalassemia patients it was (166.23 ± 8.45 U/ml) compared to the control group that, recorded (81.06 ± 26.79 U/ml), may be the reason for the high concentrations of liver enzymes due to very high levels of ferritin that collects primarily in the liver shows its negative effects on the liver, enzymes clearly (4,17).

A slight positive relationship has been observed between serum ferritin with hepatic enzymes and hemoglobin in thalassemia patients, as high levels of hepatic enzymes are related to some extent to the concentration of ferritin in the serum, or because of the need to build peptide chains through the effectiveness of this enzyme in the formation of amino acids required for construction (16).

The GOT enzyme is more effective than the GPT enzyme. The first enzyme is more common in the tissue of the heart, liver, and skeletal muscles as well as the kidneys, while the liver contains large amounts of the GPT enzyme, and other tissues such as the kidneys, heart, and skeletal muscles contain abundant amounts of this enzyme, possibly due to the amounts of iron present in patients’ serum, which is deposited in these organs, which results, in the breakdown of the fat of some cells of these organs (3).

As for the ALP enzyme, the research results showed a significant increase in the activity of this enzyme compared to the enzyme activity in the healthy serum.
The reason for this may be due to; the fact that most of the activity of this enzyme comes from bone tissue and, since thalassemia patients suffer from the dissolution of this tissue, this leads to the leakage, of this enzyme into the blood circulation, and then an increase in the effectiveness of this enzyme (15).

Iron is one of the essential minerals that the body needs to complete the formation of erythrocytes, oxidative metabolism, and cellular immune response, and plays, an essential role in many body functions. Iron is the site of oxygen binding in Heme-containing proteins such as, hemoglobin and myoglobin, (18). Iron is absorbed by the gut and transported to the blood by a special protein called Transferrin, as the receptors for this carrier protein are found on; bone marrow cells and some other cells of the body, and the ability of this protein to bind to iron increases, in the event of high iron consumption by the body (15).

Iron deposition in the liver, spleen and bones resulting from the blood transfusion, process may cause distortions in the shape of the body and its deposition in the organs that we have mentioned, which leads to their enlargement (5). Therefore, this requires working to displace iron to reduce complications, by giving the patient medications to remove iron from the body and other drugs such as folic acid, Vitamin C, including desiferal injections, is injected under the skin for five days a week, in addition to another treatment, Exjade pills, given daily once, according to the child’s weight, given, his dose of (15 mg per kilo - 40 mg per kilo) depending on the patient’s weight and, high iron content. In His Blood (19,20).

Conclusions and recommendations:

Conclusion

We conclude from this study:

1. The higher incidence of thalassemia in males than in females

2. The incidence of infection decreased, with age, and it peaked in the age group (2-6) years and decreased to the lowest, levels in the age group (22-26) years.

3. There is a decrease in hemoglobin concentration and high serum ferritin concentration in people with thalassemia, compared with healthy people.

4. There was an increase in the levels of hepatic enzymes (GPT, GOT, ALP) in patients, compared to healthy levels.

5. Deposition, of iron in patients’ bodies adversely affects liver function.

6. There is a positive correlation between serum ferritin and liver enzymes in thalassemia patients.

Recommendations

1. Intensifying awareness campaigns through various media such as newspapers, magazines, television and lectures and publishing, brochures on thalassemia as it is a genetic disease that may be limited by proper planning and avoiding marriage of relatives as much as possible in families with a history of the disease.

2. Specialized, centers shall be determined to work on finding a formula for recording the numbers, of the injured, their addresses and the sick, history of the family, from grandparents to parents. And the establishment of centers specialized in bone marrow transplantation as part of the, treatment requirements for thalassemia.

3. Emphasis on conducting a pre-marriage screening test for thalassemia, and adding it to the list of examinations, conducted for this purpose may help with medical advice, before marriage.

4. Emphasis on prenatal diagnosis, especially in families where the disease appears frequently.

5. Urging families with a history of thalassemia to determine childbearing in order to reduce the frequency of infection, in the same family.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding
References


Histo-morphomertic, Histochemical Solidity Ruling of the Small Intestine in Hamsters and Rabbit According to Different Food

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Abstract

In the present study, the histo-morphometric comparison study of small intestine in hamsters and rabbit was aimed. Ten of clinically healthy animals of both sexes were collected by Al-Simawa city, age ranged between 6 month to 1 year, which was estimated according to the dental equation of the animals. The small intestine was cut and stained alternatively with Hematoxylin and Eosin (H & E) and (Lilles Alchrome stain).

The current study aims to discover the structures and measurement of small intestine belonging to these animals specifically their histo-morphometric textures. The anatomical studies revealed that small intestine of both animals consists of: duodenum, jejunum and ileum. Histological exam was showed that the wall of small intestine composed of four layers including: mucosa, submucosa, muscularis and serosa and adventitia.

Key words: histology, small intestine, rats, duodenum, epithelium.

Introduction

The small intestine was observed to be divided into (duodenum, jejunum and ileum). The duodenum was long and started at the pylorus region close to the stomach. The jejunum appeared to be convoluted or coiled, very long and occupied the abdominal floor between the stomach urinary bladder. The ileum was short and followed the jejunum and marked the end of the small intestine. The distal end of the ileum had a thick walled enlargement, Sacculus rotundus which mark the junction between the ileum, cecum and colon (1,2).

The major functions of the small intestine are digestion, secretion, and absorption. The small intestinal mucosa has several anatomic adaptations that serve to create an immense surface area with which to digest and absorb nutrients. These include the plicae circulares (intestinal folds), villi, and microvilli. The small intestinal mucosa is characterized by numerous, regularly distributed luminal papillary projections called villi. Villi are lined by columnar epithelial cells, enterocytes. Enterocytes have apical microvilli (brush border) (3).

The chyme passes into the small intestine from the stomach. Flow into the small intestine is regulated by the pyloric sphincter. The small intestine is the place where the majority of digestion and absorption of nutrients occurs. The small intestine can be divided into three sections – duodenum, jejunum, and ileum (4).

The duodenum is the first section and is the site of most digestion. Buffers are secreted from the pancreas into the small intestine to increase the pH to a more neutral level, as the stomach acids would make the digested food have a low pH level. In addition, a variety of enzymes are secreted by and into the small intestine to break down the food. These include proteolytic, lipolytic (fat digesting), and amylolytic (starch digesting) enzymes (5,6).

The jejunum is the middle section of the small intestine. Many nutrients, such as amino acids, fatty
acids, and glucose, are absorbed here. The last section of the small intestine is the ileum. In the ileum, the remaining digested nutrients are absorbed, as well as the B vitamins (7).

The ileum is mainly to absorb vitamin B\textsubscript{12} and bile salts and whatever products of digestion were not absorbed by the jejunum. The wall itself is made up of folds, each of which has many tiny finger-like projections known as villi on its surface. In turn, the epithelial cells that line these villi possess even larger numbers of microvilli. Therefore, the ileum has an extremely large surface area both for the adsorption (attachment) of enzyme molecules and for the absorption of products of digestion (8).

### Materials and Methods

#### Animal source

A total of ten mature animals of both sexes (5 hamsters and 5 rabbits) were used for this study. They were purchased from local market of Al-Samawaa city, Al Muthanaa government, Iraq. The animals were transported using laboratory hamsters cages to the department of veterinary anatomy laboratory, University Al-Muthanaa, Iraq.

The animals were acclimatized for three days prior to the research and had free access to elephant grass, commercial feed supplement and water. The animals were observed to be in good nutritional status on physical examination before euthanasia. They were all sedated using gaseous chloroform in a confined container and later sacrificed.

#### Gross anatomy

For each animal, an incision was made on the ventral midline immediately after sacrifice and the abdominal cavity was exposed. The small intestines were dissected from the mesenteric and spread in a straight line. The gross anatomical structures of the entire intestine were observed.

Photomacrographs were taken using a digital camera. Tissue samples were collected from the segments of the small intestine (Duodenum, Jejunum and Ileum) and fixed 10% in buffered formalin for histology.

#### Histology

The fixed tissues (duodenum, jejunum and ileum) were cut into blocks and identified. They were then dehydrated through a series of graded alcohols (70%, 80%, 90%, 95% and 100%). The blocks were cleared in xylene and then infiltrated with molten paraffin wax. Sections (6 \mu m) microns thick were cut from embedded tissue using (Jung Rotary Microtome (model 42339)) (9).

The tissues were then mounted on grease free clean glass slides. The slides were prepared at room temperature stained alternatively with Hematoxylin and Eosin (H & E) and (Lilles Alchrome stain). The prepared slides were studied using light microscope (Olympus binocular microscope). Photomicrographs of the prepared slides mounted on the binocular microscope were taken using a digital microscopic objective. These pictures were then transferred to a computer and detailed studies were carried out (10).

### Results & Discussion

#### Morphological aspect: The small intestine of animal studies were distinctly divided into three segments, namely the duodenum, jejunum and ileum. The three grossly divided parts of small intestine in the current studied animals were similarly observed in other studies such as (11–13).

The duodenum consisted of descending and ascending limbs forming U-shaped tube called duodenal loop. The pancreas observed between these limbs. The U-shape of duodenum in the current animals was commonly observed in the other (this result disagreement with other studies such as Agur et al (14) those said the duodenum it was found to form an “S” shaped curve and also the (15,16) mention the duodenum it found is a C-shaped or horseshoe-shaped structure.

The jejunum of the animals was organized grossly in the form of cone-shaped of spiral coils. The cone had centripetal coils, a sigmoid flexure and centrifugal coils. This jejunum shape was similar to other studies in squirre (17).

The third segment of the small intestine of the animals was the ileum which appeared the shortest part of the small intestine. It joined the jejunum cranially and extended caudally to join the cecum these result
agreement with other studies such as (14,17).

**Histological aspect**

The small intestine appeared long convoluted tube, extends from the junction with stomach to joining point with the large intestine. Three segments were found in the small intestine of the studied animals, namely duodenum, jejunum and ileum these result agreement with other studies such as (15,17).

**Duodenum:** The organ showed microscopically the four classic known layers of a tube organ: mucosa, submucosa, muscularis and serosa (Fig.1). These four layers appeared in the duodenum and other parts of the small intestine in all such as (18,19).

**Tunicae mucosa:** The duodenal mucous membrane in the animals showed three different parts (Fig.2), that were lining epithelium (simple columnar cells) (Fig. 2), lamina propria (loose connective tissue with the presence of mucosal glands) (Fig.2,1 ) and muscularis mucosa (two thick layers of smooth muscle arranged into inner circular and outer longitudinal bundles). The presence of two layers of muscularis mucosa in the duodenal mucosae of animals was similar to the findings observed in (20). The mean thickness of this tunica in hamster was 2131.2mm, whereas, in the rabbit was 2410.1 mm.

**Duodenal villi:** They were finger-shaped mucosal projections which constructed from the lamina propria, smooth muscle fibers as well as the lacteal. The latter was blind ended lymphatic capillary that is lined by simple columnar epithelium in studied animals (Fig.1,a) these result agreement with other studies such as (2,21).

The lining epithelium of the villi was similar to those observed previously in the same organ these similarity with study (3). The irregularity that observed in the mucosal surface could be due to the presence of duodenal villi intervening between the bases crypts of Lieberkühn. The mean length of villi in hamster was 7251.3mm, whereas, in rabbit about 9764.7 mm.

**Duodenal Crypts of Lieberkühn:** These were simple tubular glands called intestinal glands that were extended from the muscularis mucosa till the bases of the villi. They were lined by a simple columnar epithelium similar to the lining epithelium of the duodenal lumen (Fig.2,a ). The mean diameter of gland in hamsters was 3228 mm and in rabbit 5783.1mm.

**Tunicae Submucosa:** It was formed irregular dense connective tissue situated, beneath the muscularis mucosa, and the layer composed of large blood, lymphatic vessels. The Brunner glands that found in submucosa in mammals concert. The mean thickness of this tunica in hamster was 1877.3mm, whereas, in the rabbit was 2124.1 mm.

**Tunicae Muscularis Externia:** Underneath submucosa the muscular coat consists of the smooth muscles fibers arranged into two layers, the inner circular and the outer longitudinal layers in the studied animals(Fig.1,a). Evenly the inner layer was thicker than the longitudinal layer over all parts of the duodenum. This finding was agreed with studies (16) in wistar rats. The mean thickness of this tunica in hamster was 4342mm, whereas, in the rabbit was 5117 mm.

**Serosa and adventitia:** The layer appeared thin in thickness constructed by loose connective tissue covered by a layer of mesothelial cells (Fig.1,a ). The serosa lined externally the muscularis. These findings were similarly recorded in other result disagreement with (4) in wistar rats. The adventitia or outer layer consists of a serous membrane composed of connective tissue covered the free part of organ. The mean thickness of this tunica in hamster was 200mm, whereas, in the rabbit was 240 mm.

**Jejunum:** The microscopic examination of jejunum’s wall showed similar histological layers of a tube organ (Fig.3,b ).

**Tunicae mucosa:** The mucous membrane was thrown into large numerous long leaf-shaped villi that were arranged in a finger like projections in animals. The epithelial lining represented by single layer of tall columnar cells of both villi and crypts in the studied animals (Fig.3,b ) which was in a good agreements with what was recorded by other result such as (4) in wistar rats. The mean length of villi in hamster was 7640mm, whereas, in rabbit about 8951mm. The crypts of Lieberkühn were short and simple tubular ducts opened at the bases of villi occupying most of the thickness of the lamina propria till the muscularis mucosa (Fig. 3,b). The mean diameter of gland in hamster was 2879mm and in rabbit was 2276mm. The lamina propria consists
of loosely packed connective tissue containing blood vessels and muscle fibers (Fig.3,b) and such finding was comparable with that observed by other studies (4) in wistar rats.

The muscularis mucosa in the animals was poorly developed and composed of only a few bundles of circular muscle fibers (Fig. 1,b). The mean thickness of this tunica in hamster was 2621 mm, whereas, in the rabbit was 2791 mm.

**Tunicae Submucosa**: The submucosa was a thicker layer of loose connective tissue possessed many blood vessels (Fig. 1,b). The mean thickness of this tunica in hamster was 1372 mm, whereas, in the rabbit was 1912 mm.

**Tunicae Muscularis Externa**: This layer was constructed of a thin outer longitudinal and a thick inner circular layers in the studied animals. Between these muscle bundles, fine dispersed narrow connective tissue layer containing many large blood vessels (Fig. 1,b). The presence of two muscular layers in the present animals was similarly recorded in other studies such as (15). The mean thickness of this tunica in hamster was 4320 mm, whereas, in the rabbit was 4771 mm.

**Tunicae Serosa**: It was formed by layer of simple squamous epithelium under which was a thin layer of loose connective tissue (Fig. 1,b). The mean thickness of this tunica in hamster was 180 mm, whereas, in the rabbit was 200 mm.

**Ileum**: Similar to the previous tube like organs the microscopic examination of ileum’s wall showed the four layers: mucosa, submucosa, muscularis and serosa (Fig.4).

**Tunicae mucosa**: The villi appeared small leaf-shaped arranged in a zig-zag pattern (Fig. 4,c). Each villus was lined by an epithelium while its center contained connective tissue core and such construction was agreed with results of studies (4) in wistar rats. The villi were short and less numerous compared to those found previously in the jejunum and duodenum of the same investigated animals. The lining epithelium was simple columnar (Fig. 1,c). The epithelium showed obviously higher number of goblet cells compared to those observed in both duodenum and jejunum. The mean length of villi in hamster was 3481 mm, whereas, in rabbit about 3632.1 mm. Loose connective tissue observed in the propria just beneath the epithelial lining (Fig. 1,c) which was similar to (17) in rats when a simple columnar epithelium supported by underlying connective tissue propria.

The muscularis mucosa was made of a thin outer longitudinal and a thick inner circular layers of smooth muscle fibers (4) in wistar rats. The mean thickness of this tunica in hamster was 1815 mm, whereas, in the rabbit was 2126 mm.

**Tunicae submucosa**: This layer was formed of loose connective tissue with blood vessels and these findings agreed with that recorded in (4) in wistar rats. The mean thickness of this tunica in hamsters was 1311 mm, whereas, in the rabbit was 1521 mm.

**Tunicae Muscularis Externa**: The layer muscularis was made up of an inner circularly and an outer longitudinally arranged layers of smooth muscles bundles (Fig. 1,c). This muscular arrangement was similar to that in (17) in rats. The mean thickness of this tunica in hamsters was 2321 mm, whereas, in the rabbit was 2853.4 mm.

**Tunicae Serosa**: Layers serosa was a thin layer of loose connective tissue. Its external surface was lined by simple squamous epithelium (Fig. 4,c). The mean thickness of this tunica in hamster was 160 mm, whereas, in the rabbit was 180 mm.
The dimensions of duodenum, Ilium nad Jejunum in hamsters and rabbit (µm) (Mean ± SE).

Fig. 1. Cross section of the small intestine wall of Rabbit showed mucosa (A), Submucosa (B), Muscularis (C), and serosa (D), muscularis mucosa (E), (a) duodenum, (b) jejunum, (c) ileum H & E, X100 (a) and (b), (c) X200

Fig. 2. Cross section of the small intestine wall of Rabbit showed: Crypts of Lieberkuhn (A), Brunner’s glands (B) connective tissue (green), (a) duodenum, (b) jejunum, (c) ileum.
Fig. 4. Cross section of the small intestine wall of hamster showed: Crypts of Lieberkuhn (A), Brunner’s glands (B) connective tissue (green), (a) duodenum, (b) jejunum, (c) ileum. Lilles Alchrome stain, X40

Fig. 3. Cross section of the small intestine wall of hamster showed mucosa (A), Submucosa (B), Muscularis (C), and serosa (D), goblet cells (E), (a) duodenum, (b) jejunum, (c) ileum H & E, X100
Lilles Alchrome stain, X40

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Awareness of Health Workers in Premature Units At Kirkuk City Hospitals Concerning Neonatal Jaundice

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Abstract

Objective: this study aimed to assess the awareness of the health workers in premature units regarding neonatal jaundice at Kirkuk city hospitals, as well as to find out the socio demographic characteristics of the study sample.

Methodology: A descriptive study was carried out at premature units of Azadi Teaching Hospital, Kirkuk General Hospital and Pediatric Hospital of Kirkuk city, for a period from the 1st February and up to the 15th of May, 2019 and to achieve the objectives of the study. A non-probability (purposive) sample of (62) health workers working at premature unit were recruited. Developed questionnaire was constructed for the purpose of the study which consisted of two parts: the demographic characteristics; awareness part (knowledge and practices). The data were collected through the use of self- administered technique. They were analyzed through the application of descriptive statistical analysis (Frequency, Percentage % and mean score) data analysis.

Results: The findings of the study indicated that (45.2%) of the staff were from pediatric hospital. (40.3%) were graduated from Nursing Institute, (43.5%) had experience from 1<5 years and (74.2%) had participated in a training course mostly inside the country. The total grand mean score for the staff awareness (knowledge and practices) were adequate (1.73).

Conclusions and Recommendations: Assessment for the study sample awareness was adequate. Educational programs should be designed to increase the health workers awareness working at pediatric departments regarding management of neonatal jaundice especially those with less than 5 years’ experience.

Keywords: Neonatal jaundice, hyperbilirubinemia, health workers, Kirkuk.

Introduction

Neonatal jaundice (NNJ) is the yellowish shading of the skin and sclera of babies because of expanded degree of bilirubin in the body. It is the most widely recognized neonatal issue requiring clinical assessment and the executives everywhere on over the world. Neonatal jaundice consider one of the most well-known discernible physical signs during neonatal period. (1)

The most cases of neonatal jaundice are physiological jaundice, which occurs in 36 hours after birth, and the baby usually in good condition and the total serum bilirubin will not be too high 12mg/dl in a full term baby. (2)

Physiological jaundice in newborn can be due to, massive erythrocyte destruction, decrease in conjugation rate and poor transformation of bilirubin. (3)

The other type of neonatal jaundice is the pathological jaundice which occurs in the first 24 hours after birth and there will be rapid elevated of total serum bilirubin 5mg/dl/day. (4) The main causes for pathological jaundice, direct bilirubin is sepsis, congenital infections and liver diseases such as hepatitis and biliary atresia, while the indirect bilirubin is hemolytic disease and hypothyroidism. (1)
The early identification of newborns who are at a greater risk of enhancing severe neonatal hyperbilirubinemia is of paramount importance to preventing damage of brain. (3) In any case, bilirubin is venomous to synapses. In the event that infant be experiencing intense jaundice, there’s a hazard of passing bilirubin to cerebrum, this condition is called extreme bilirubin encephalopathy.

Neonatal jaundice (NNJ) influences up to 84% of term infants and is the most inescapable reason for emergency clinic readmission in the neonatal stage. (7) In child, jaundice will in general create as a result of two factors, the breakdown of fetal hemoglobin and the moderately youthful hepatic metabolic way ways which can’t form thus emission of bilirubin is as fast as a grown-up, this explanation an aggregation of bilirubin in the blood prompting the indication of jaundice. (8)

The known risk factors for the occurrence of infant jaundice incorporate an ABO or Rh factor contradiction among mother and infant, a newborn child of a diabetic mother, premature liver functioning, Glucose 6 Phosphate dehydrogenase deficiencies and incorrect latch of newborn to breast during breastfeeding. (9)

According to a report by Global Burden of Disease GBD in 2016, insufficiency of offices for fast, routine bilirubin assurance or imperfect irradiance (<8–10 μW/cm²/nm) from ineffectively kept up phototherapy apparatuses are levels of deferral for powerful mediation that bring about higher midpoints of avoidable and conceivably destructive trade bonding likewise bilirubin instigated mortality in creating nations. It likewise watches home conveyances do assume a part in late location and treatment of neonatal jaundice. (10) It is known United Nations Children’s Fund (UNICEF) intents to decrease in death rates occurring at neonatal age, Global Burden of Disease (GBD) indicates that first trial to estimate the burden of severe NNJ assessed to influence 481,000 late-preterm and term children yearly, with 114, 000 biting the dust and >63, 000 getting by with moderate or extreme long-haul neurologic weaknesses. (10, 11) That’s why we need to further research and interventions to put the alarming problem under monitoring.

This investigation was directed to distinguish the information and practices of the nursing staff who are working in the premature unit in three hospitals in Kirkuk city regarding the neonatal jaundice.

**Methodology**

**Design and setting of the Study:** An engaging report was done from February, 1st, 2019 to May, 15th, 2019 so as to accomplish the targets of the current examination. The examination was directed in untimely units of three medical clinics (Pediatric Hospital, Azadi Teaching Hospital and Kirkuk General Hospital) in Kirkuk city.

**Test of the examination:** Non - Probability inspecting approach (purposive example) comprises of 62 health workers working at premature units of the three hospitals.

**Inclusion Criteria:** Only health workers who were working at premature units of the three hospitals.

**Instrument Construction:** For the purpose of the present study, a questionnaire format was constructed to assess the health workers awareness (knowledge and practices) regarding neonatal jaundice. The poll design depended on the audit of writing and related past investigations. Formal consent was obtained from the nursing staff who agreed to participate in the study, the questionnaire was self-administer and took 10-15 minutes to be complete. The investigation instrument included three sections, which was comprised of the accompanying factors: The investigation instrument included three sections, which was comprised of the accompanying factors:

1. **Part One/Socio-segment qualities:** which includes: Type of hospital, Sex, Educational level, marital status, residence, Socio-economic status, years of experience in premature unit, participation in a training course, period of the training course and the place of the training.

2. **Part Two/ Assessment of awareness (knowledge part):** Include 15 items regarding knowledge of the sample. It has been consist of two scales as two (2) for ‘‘Yes’’ and one (1) for ‘‘No’’.

3. **Part Three/ Assessment of awareness (practice part):** Include 7 items regarding practices of the study sample.
Legitimacy of the examination scale: To guarantee the legitimacy of the scale, technique and system were proposed to be done during the investigation. Nine specialists of various fortes identified with the field of the current investigation were picked to survey face and substance legitimacy. They were approached to audit the scale design for lucidity and ampleness so as to accomplish the current examination goals. Certain changes were utilized dependent on the specialists’ proposals and recommendations.

Pilot study: The pilot study was completed for the period from February, 15th, 2019 to February, 30th, 2019. A pilot study was conducted before starting actual data collection, time need to complete each questionnaire and self-administered ranged approximately (10-15) minutes. Selected 4 staff from pediatric Hospital, 3 from Azadi Teaching hospital and 3 staff from Kirkuk general hospital. The sample of the pilot study were excluded from original sample of the study. The purpose from the pilot study was to recognize the obstructions that might be experienced during information assortment, gauge the time required for information assortment and to make certain of the precision of the scales.

Strategy for Data Collection: Data were gathered through self-administered technique. The examiner had exhibited targets and the centrality of examination and the advantage of the investigation to the member. Verbal consent obtained from premature unit nursing staff. Each nurse spends approximately (10-15min) to respond to the questionnaire.

Period of Information Collection: Data assortment has been directed during a time of two months stretching out from February 15th, 2019 to April 15th, 2019.

Statistical analysis: Data were analyzed in several steps. First, descriptive statistics, which includes frequency and percentages, and Mean score. Data were arranged, composed and went into the PC record; Statistical Package for Social Science (SPSS) (20 rendition) is utilized for information examination at (P. esteem ≤ 0.05). Information were analyzed through the application of two approaches:

Descriptive statistical data analysis: This approach is employed through:

- Frequency distribution
- Percentage (%)

\[
\%	ext{ } = \frac{\text{Frequencies}}{\text{sample size}} \times 100
\]

- Mean of Scores

This calculation is applied for the assurance of thing’s centrality of the evaluation apparatus comparative with every angle. Cut-off points are used for this determination low-significant, moderate significant, and highly significant.

\[
M . S = \frac{f_1 \times \text{score}_1}{n_1} + \frac{f_2 \times \text{score}_2}{n_2}
\]

It is computed as follows:

\[
M . S = \text{Mean of score}
\]

\[
f = \text{Frequency}
\]

\[
n = \text{Number of cases}
\]

So the cutoff point was calculated according to the following formula

\[
\frac{1+2}{2} = 1.5
\]

A mean of score of (1- less than 1.5) was considerate inadequate and from (1.5 - 2) was considerate adequate

Rating and scoring of the scale: The mindfulness (information and practices) poll things were appraised and scored to things as, (2) for Yes, and (1) for No. Data of the study were ordinal according to two levels scale of (Yes, No) which were scored as (2, 1) for each level respectively.

Ethical approval: Ethical approval were obtained from Kirkuk Directorate of Health as well as from the moral council in the school of nursing, Kirkuk University.

Results

The present study sample were distributed to the three hospitals as follows, (45.2%) of them were from Pediatric Hospital, (32.3%) were from Kirkuk General
Hospital, while (22.6%) of the participants were from Azadi Teaching Hospital. (figure 1)

Figure 1: Distribution of the study sample

Regarding to sex, (79%) of the participants were female, while only (21%) were male, according to the educational level (40.3%) of the participants were graduated from nursing institute, while (11.3%) of them had graduated from nursing college.

According to marital status, (50%) of study sample were married, while (6.5%) were divorced and widow. While to residence, (82.3%) were from urban and (17.7%) were from rural. socio-economic status, (58.1%) had barely sufficient economic status and (27.4%) had sufficient economic status.

Regarding to years of experience in pre mature unit (43.5%) of participants had (1<5) years’ experience, while (1.6%) of them had (15<10) years of experience, while according to participation in a training course (74.2%) of them answered yes, while (25.8%) answered No, and those who participate in a training course less than one week were (46.8%), while (24.2%) had from (1week<11month) training course, and (71%) of these training courses were inside Iraq.

Table 1: Shows the knowledge of the study sample regarding neonatal jaundice

<table>
<thead>
<tr>
<th>NO.</th>
<th>Knowledge part</th>
<th>YES</th>
<th>NO</th>
<th>MS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Jaundice is increasing of bilirubin in the Blood</td>
<td>61</td>
<td>1</td>
<td>1.6</td>
<td>Adequate</td>
</tr>
<tr>
<td>2</td>
<td>Bilirubin is pigmentation consist due to breaking RBC</td>
<td>55</td>
<td>7</td>
<td>11.3</td>
<td>Adequate</td>
</tr>
<tr>
<td>3</td>
<td>Neonatal jaundice is common in neonate and disappear without complication</td>
<td>39</td>
<td>23</td>
<td>37.1</td>
<td>Adequate</td>
</tr>
<tr>
<td>4</td>
<td>There are 2 types of jaundice, physiological and pathological</td>
<td>55</td>
<td>7</td>
<td>11.3</td>
<td>Adequate</td>
</tr>
<tr>
<td>5</td>
<td>Neonatal jaundice occurs mostly in first week of life</td>
<td>55</td>
<td>7</td>
<td>11.3</td>
<td>Adequate</td>
</tr>
<tr>
<td>6</td>
<td>Neonatal jaundice occurs between 3rd and 4th day in full term babies</td>
<td>49</td>
<td>13</td>
<td>21.0</td>
<td>Adequate</td>
</tr>
</tbody>
</table>
physiological jaundice occurs due to increase in Unconjugated bilirubin

Pathologic jaundice appears due to increase destruction of RBC

Neonatal jaundice appears due to Rh Incompatibility

G6PD can be one cause of Neonatal jaundice

Blood diseases can be a cause for Neonatal jaundice

Birth defects and infection can be a cause for Neonatal jaundice

Increasing of bilirubin can cause Kernicterus

Severe jaundice can cause convulsion

Severe jaundice can be fatal

Table (1) shows the general knowledge of the study sample regarding neonatal jaundice. The result found adequate knowledge for the nursing staff regarding neonatal jaundice and the grand mean score for knowledge part was (1.72).

Table 3: shows the practice of the study sample regarding neonatal jaundice
Table (2) shows the participants practice regarding neonatal jaundice. The result found adequate practice of the nursing staff regarding neonatal jaundice, except in item (1) which was “Most of N jaundice does not need treatment” and item (2) which was “Follow the baby without admission” were inadequate (MS: 1.4). The total grand mean score for the practice part was (1.74).

Table 3: shows the total grand mean score for the study sample awareness

<table>
<thead>
<tr>
<th>Assessment</th>
<th>GSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>1.72</td>
</tr>
<tr>
<td>Practice</td>
<td>1.74</td>
</tr>
<tr>
<td>Total</td>
<td>1.73</td>
</tr>
</tbody>
</table>

While table 3 shows the total GMS for the study sample knowledge and practices, the result found that the knowledge and practices of our study sample were adequate.

Discussion

The finding of the current examination showed that most of the investigation test (79.0%) was female. The current study is in agreement with a study by Khudhair S, (2016) (12) of effectiveness of health educational program upon nurses practices toward care of newborn regarding neonatal jaundice, which found that the majority of the study sample were female, and this can be explained as the majority of females had cozy toward care baby so they like to work in pediatric medical clinics. Moreover, most of the sample graduated from the institute of nursing. Our result was in agreement with a study by Khudhair S, (2016) (12) who referenced that the majority of the investigation test were moved on from the clinical foundation in his examination about adequacy an instructive program upon medical attendants rehearse toward care of infant with respect to neonatal jaundice. In the current investigation around two third of health workers live in metropolitan zone and half of the nurses were married, while (37.1%) of them were single. The results were in the same line with Ahmed et al (2017) (13) who observed that most of them were married. Comparable to the attendants’ long stretches of encounters in the neonatal emergency unit more noteworthy level of them had 1<5 years’ understanding and accounted (43.5%) while only (1.6%), (6.5%) of them had experience from 15 to more than 20 years. Regarding to participation in a training courses, around three quarters of the sample have involved in a training course of being less than one-week period the majority (46.8%) and most of the training courses was inside the country. This finding is upheld by Watson(2011)(14) who referenced that all members the length of administration extended from 1 to 5 years. The result by Khudhair S, (2016) (11) also disagree with the current study as the medical attendants’ long stretches of encounters in the neonatal emergency unit more prominent level of them had 12-16 years’ understanding and accounted (36.7%) and (40.0%) of them had over 17 years of encounters in nursing. The attendant assumes fundamental function to guarantee the norm of care for all babies so as to restrain intense bilirubin encephalopathy and kernicterus and the medical caretakers should fill in as a crew to guarantee that all newborn children are screened for of danger raised bilirubin levels (hyperbilirubinemia) preceding clearing from the emergency clinic. Awhonn S.et.al. (2009) (15). As regard to the knowledge of the health workers in premature unit of the three hospitals, the current study found that the assessment for the participant’s knowledge regarding neonatal jaundice was adequate knowledge for all items with grand mean score (1.72). This result was in agreement with the result of Adebami, (2015) (16) who found that there was significantly better understanding of neonatal jaundice causes such as blood group incompatibility, G6PD deficiency, low birth weight and infection among optional medical services laborers than essential consideration laborers (p < 0.007). Similarly, secondary health care workers were
better informed on appropriate approach to management of neonatal jaundice like the need for referral, laboratory tests to determine the causes and severity, possible treatment options like phototherapy and/or exchange blood transfusion when severe (p=0.000). Regarding the health workers practices in premature unit of three hospitals, the result showed that the participants practice were adequate, except in item (1) which was “Most of neonatal jaundice does not need treatment” and item (2) which was “Follow the baby without admission” were inadequate (MS: 1.4) respectively. The total grand mean score for the practice part was (1.74). A study by Abai G.et al.(2011) (17) has found that intercessions taken assisted with advancing the information and practice of prescribed measures to unveil neonatal jaundice early. More grounded accentuation must be put on utilizing the new revealing strategies and new nursing sheets. Offices of vehicles for all bustling maternal and kid wellbeing centers for home nursing consideration is enthusiastically suggested.

Conclusions and Recommendations: Assessment for the study sample awareness was adequate. Educational programs should be designed to increase the health workers awareness working at pediatric departments regarding management of neonatal jaundice especially those with less than 5 years’ experience.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

Study of Some Immunity Characters Results of Injection antigenic *Staphylococcus sp.* in Local Rabbits

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Abstract

This study was conducted to identify some immune manifestations resulting from the injection of staphylococcal bacterium antigens Heat-killing with a concentration of 0.75 ml in muscle and concentration 0.5 ml subcutaneous in local rabbits to see how immune response of these antigens achieve. Tests were carried out (late hypersensitivity, phagocytosis of candida albicans, and NBT dye reduction).

The results showed that the highest level of skin thickness was 9.50 ml compared to control 1.48 ml after 24 hours of antigen injection. The results also showed that the highest level of candida yeast is 81.30% when injecting concentration 0.5 ml under the skin compared to control 79.33%. As for the reduction of NBT dye, the highest level of dye reduction was 14.70% when the concentration was injected 0.75 ml with the muscle compared to 10.63% control.

We conclude that the injection of staphylococcus antigens (killed by heat) has the potential to induce immune response in local rabbits

Keywords: *Staphylococcus*, Immunity, Rabbits

Introduction

*Staphylococcus* is considered a cluster bacterium for its non-moving cluster shape and discolored according to gram, an anaerobic bacterium optional with the exception of sheep’s Staph. *Aureus*. which is obligatory anaerobic (¹). It currently has approximately 20 or more types of skin injuries such as daisies and lobes and has a high pathogenic characteristic and contains an important pathogenic factor, coagulase. Bacteria are considered one of the most famous factors that cause the infection because of its wide spread spectrum, because of the range of infection from simple to serious, as these bacteria have the potential to spread the infection widely and its toxic effect(²–⁴).

The aim of the research is to determine the extent of the immune response against staphylococcus antigens in local rabbits.

Material & Methods

Sampling and bacterial isolation:

Swab samples were collected from inflamed wounds in animals and then grown on three culture transplant and it’s:

- Nutrient Agar
- Blood Agar
- MacConky Agar

Then placed in the incubator for 24 hours with a temperature of 37.2 c0 and then observed the growth of bacterial colonies on the implant circles, the eye was taken from the colonies and dyed with gram stain and upon examination it was found positive for this dye and it is a cluster bacteria (cocci).

To find out the sex of these bacteria, he took his eye and grew up on another medium, Mannitol Salt Agar, a
special medium for the growth of Staphylococcus, placed in the incubator with a temperature of 37.5 °C for 24-48 hours observed the growth of bacterial colonies. He then attended a liquid transplant, Brain Heart Infusion Broth, for mitigation purposes. After preparing the medium we planted in one of the tubes bacteria Staphylococcus and then put in the incubator and after 24 hours observed the change of the middle from net to turbidity indicating the growth of bacteria then attended 6 tubes of the liquid implant center in each tube 9 ml to perform the mitigation process. He took 1 ml of the tube containing on a bacterium and then put in the tube number 1 and then 1 ml of tube 1 and put in the tube number 2 and then 1 ml of number 2 and put in number 3…. Etc. To tube 6 where we took 1 ml and we neglected it. Then he took from each of the tubes (4-5-6) one ml (1 ml) and the other 0.1 ml and put in the dishes of the implant and then poured the center of the implant on top of it and then we moved each dish 25 turns clockwise and 25 cycle counterclockwise for the purpose of mixing bacteria with the culture media. Incubated the dishes were heated at a temperature of 37.5 °C and for 24-48 hours after which we found the growth of bacteria in the implant editing circles where the growth in the two concentrations for tubes 4-5 was very dense (colonies cannot be counted) as well as for the concentration of 1 ml for tube 6. As for the concentration of 0.1 ml for tube 6, the number of colonies was 242 colonies by a growing bacteriological on the plate (the colonies were calculated by the colony counting device), by equation. (Number of colonies * inverted dilution * solution size).

When calculating concentration at 1 ml, it was found to be =242*10^7 colony by bacteria colony/ml. Then killed the bacteria by putting them in a water bath temperature of 100 °C for half an hour, then they took sample and grew on the culture media to make sure that the bacteria were killed and after the incubation for 24 hours /37.5 °C did not grow the bacteria(5).

2- Injection of dead bacteria into rabbits:

After killing the bacteria, take three groups of local rabbits in the animal field of the Faculty college of Veterinary Medicine in Fallujah university, took each group of three rabbits that injected the first group with a concentration of 0.5 ml subcutaneous, and injected the second group with a concentration 0.75 ml with the muscle and left the third group for control. After 14 days of the first injection, it was injected with the same concentrations again and a week later the blood was drawn for work immunological experiments.

3- Immunological tests

A- Hypersensitivity Test

Two groups of local rabbits belonging to the Faculty of Veterinary Medicine in Fallujah took each group of thirteen rabbits injected the first group with staphylococcus bacterium antigens killed by heat with a concentration of 0.1 ml inside the skin, and left the second group to control. The thickness of the skin and the diameter of the red circle were measured in the infected group and in the control group after 24-48-72 hours of injection.

B- Isolation of polymorph nuclear neutrophils cell from the blood.

Blood was drawn from the group of infected people and from the control group from the heart by a wine syringe and placed in sterile plastic tubes and sealed container on heparin with a concentration of 500 global units to prevent coagulation and dextran solution to degenerative red blood cells and then gently blended the contents of the tube. The tubes were then placed in the incubator warmly 37 °C for 45 minutes, after which the plasma layer containing the white blood cells was withdrawn and transferred to another sterile plastic tube. White blood cells were washed with hanks local balanced solution twice by discarding them at 1500 rpm to remove plasma and heparin. The cells were then suspended in the same solution, with a concentration of 1*10^6 cells/ml(6).

A- The effect of staphylococcus on the reduction of Nitroblue tetrazolium stain (NBT for PMNs cells).

Put 0.75 ml of PMNs cell stuck in silicone-coated glass tubes and then add 0.75 ml of NBT dye prepared according to the method(7) the tubes calmly spin the hand to mix the contents of the tube with the provisions of the provisions placed the pipes then in the incubator temperature 37°C for 25 minutes. After the end of the incubation period he took 20 microliters from each tube to a glass slide and spread gently and then left to dry and fixed with methanol and then dyed your dye as a
muzzle prepared according to the method of Allen and his group\(^{(8)}\) for 15 minutes after which 200 cells under the optical microscope 100 X were calculated for the purpose of extracting the percentage of cells that were able to produce superoxide ion and thus reduce the yellow dye NBT to the blue furor granules that deposit in the eye-cell\(^{(7)}\). After the end of the incubation period he took 20 microliters from each tube to a glass slide and spread gently and then left to dry and fixed with methanol and then dyed by Geimsa stain prepared according to the method of Allen and his group\(^{(8)}\) for 15 minutes after which 200 cells under the optical microscope 100 X were calculated for the purpose of extracting the percentage of cells that were able to produce superoxide ion and thus reduce the yellow dye NBT to the blue furor granules that deposit in the eye-cell\(^{(7)}\).

A- The effect of Staphylococcus infection on the heat-dead candida yeast:

· Preparation of AB blood group

Pull 10 ml of the blood of a healthy people AB and put it in a sterile plastic tube and leave at room temperature for 30 minutes and then centrifuge quickly 2000 cycle/minute for 15 minutes, pull the top layer containing the required material (antibodies and complement), and save at a temperature \(-20^\circ\)C until use.

· Phagocytic examination

Placed in sterile plastic tubes 0.25 ml of PMNs cell that concentration \(1\times10^6\) cells/ml added 0.25ml of heat-killed yeast cells prepared by method (Wilkinson, (1977), by 1 cell PBNs: 4 killed yeast cells It was added 0.25ml of AB human blood group, after which the tubes were incubated at a temperature of 5-10% Co2 for periods (30-60-90-120) minutes where they prepare a tube for all time and for all trials (Harry etal., 2014). Then I made four slides for each tube left to dry and fix methanol for two minutes and then dyed with a pigment giemza for 15 minutes calculate 200 phagocytic cells and not phagocytic cells using optical microscope zoom 100 X to calculate the pharynx coefficient =

\[
\text{Pharyngeal coefficient =number of pharyngeal cells/total pharyngeal cell number (phagocytic cells + not phagocytic cells) x 100}^{(6)}
\]

Results

Table 1 shows late hypersensitivity reactions when staphylococcus antigens are injected into the skin and have the highest value for skin foldfish when heat-killing antigens are injected at 0.1 ml in the skin 9.50 mm at a time of 48 hours compared to control 1.48 mm, and the highest red diameter rate was 3.54 mm at a time of 24 hours compared to control (zero).

<table>
<thead>
<tr>
<th>R</th>
<th>CONTROL</th>
<th>R</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.54</td>
<td>0</td>
<td>8.60</td>
<td>1.53</td>
</tr>
<tr>
<td>0.89±</td>
<td>B</td>
<td>1.15±</td>
<td>0.14±</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>A</td>
<td></td>
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<tr>
<td>3.26</td>
<td>0</td>
<td>9.50</td>
<td>1.48</td>
</tr>
<tr>
<td>0.83±</td>
<td>B</td>
<td>0.92±</td>
<td>0.13±</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>2.41</td>
<td>0</td>
<td>6.17</td>
<td>1.54</td>
</tr>
<tr>
<td>0.19±</td>
<td>B</td>
<td>0.83±</td>
<td>0.15±</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

Different capital letters indicate that there are significances differences between different times below the level \((p<0.05)\).
The results showed that the highest level of PBNs cell activities on the candida yeast aunt in the injury was 81.30% when the concentration was injected 0.75ml into the muscle compared to control 79.33%, although the lowest value of the candida yeast aunt was recorded 65.50% compared to control 68.66% and there were significances differences at this time, show table 2.

Table 2: Shows the effect of injecting heat-killing Staphylococcus antigens with a concentration of 0.75ml in the muscle on the candida yeast aunt.

<table>
<thead>
<tr>
<th>120</th>
<th>90</th>
<th>60</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>64.66</td>
<td>79.33</td>
<td>78.66</td>
<td>68.66</td>
</tr>
<tr>
<td>3.84±</td>
<td>2.33±</td>
<td>3.38±</td>
<td>2.33±</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>71.20</td>
<td>81.30</td>
<td>77.80</td>
<td>65.50</td>
</tr>
<tr>
<td>1.46±</td>
<td>0.23±</td>
<td>1.00±</td>
<td>2.11±</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>A</td>
<td>C</td>
</tr>
</tbody>
</table>

Different capital letters indicate that there are significances differences between different times below the level (p<0.05).

Table 3: shows the effect of injecting heat-killing Staphylococcus antigens with a concentration of 0.5ml under the skin on the candida yeast aunt.

<table>
<thead>
<tr>
<th>30 min</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>64.66</td>
<td>79.33</td>
<td>75.33</td>
</tr>
<tr>
<td>3.84±</td>
<td>2.33±</td>
<td>6.69±</td>
<td>2.33±</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>TREATED</td>
<td>69.73</td>
<td>73.83</td>
<td>79.00</td>
</tr>
<tr>
<td>0.88±</td>
<td>2.37±</td>
<td>2.08±</td>
<td>4.40±</td>
</tr>
<tr>
<td>B</td>
<td>AB</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

Different capital letters indicate that there are significances differences between different times below the level (p<0.05).

As for the reduction of NBT dye by PMNs cells, the highest percentage was 14.70% when injecting the antigen at a concentration of 0.75 ml in the muscle compared to control 10.63%, while the reduction of NBT dye was 12.43% when injecting the antigen at a concentration of 0.5 ml under the skin, Show table 4.
Table 4: shows the effect of injecting heat-killing Staphylococcus antigens with a concentration of 0.75ml in the muscle and 0.5ml subcutaneous concentration on the reduction of NBT pigment.

<table>
<thead>
<tr>
<th>IM</th>
<th>SC</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.70</td>
<td>12.43</td>
<td>10.63</td>
</tr>
<tr>
<td>0.52±</td>
<td>0.46±</td>
<td>0.65±</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>

Different capital letters indicate that there are significances differences between different times below the level (p<0.05)

**Discussion**

The results of the study showed that the highest diameter of the redness circuit was 3.54 mm while the highest rate of skin fold thickness was 9.5 mm when injecting 0.1 ml of staphylococcus spp antigens. In the skin at the time 24 and 48 hours in respectively and then start the area of the irritated area and the thickness of the skin folds gradually decreased and there were significances differences between the transactions and its control. These results are consistent with his findings(9) When injecting antigens of the weakened pseudomonas aeruginosa bacteria as well as consistent with the study(9) On the heat-weakening E. coli bacteria, they found that the injection of antigens of these bacteria led to the significances rise in both the thickness of the skin and the area of the irritated area in the local rabbits to 3.21 mm and 4.25 mm after 24 hours of antigen injection respectively. Table 2 shows the susceptibility of PMNs cells to the aunt of candida yeast in the vitro, as the results showed that the highest level of the aunt of the candida yeast aunt in the totals of transactions injected with antigen 81.3% compared to control 79.33% and there were no significant differences between the injected groups and control groups. As for the reduction of the dye of Nitro blue tetrazolium (NBT), the results showed that there are significant differences in the injection of antigens compared to non-treated control and the highest reduction rate of 14.70% when injecting antigens at a concentration of 0.75 ml in the muscle compared to control 10.6% these results are consistent with the findings(10) which stated that the rates of pharyngeal coefficient in mice treated with polysaccharides fatty to klebsiella pneumoniae bacterium due to the ability of these antigens to stimulate the components of the complementary system, especially c5a and c3b, which are involved in the process of opsonization and attracting the neutrophilic cells to the site of the infection respectively(11).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


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Correlation between Women’s Attitudes and Abstain (Used/Unused) Contraceptive Methods at Primary Health Care Centers in Baghdad City

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Abstract

**Background:** family planning is a preventive service that allows married couples achieving their desired number of children and deciding the spacing of pregnancies according to their economic opportunities and personal wishes, and to ensure that the births are at appropriate intervals for the mother and child health.

**Objective:** To find out the correlation between women attitudes and abstain and to find out the relationship between demographic characteristics and obstetrical history.

**Methodology:** A descriptive and analytic (cross sectional) study, was started at 16th September 2019 to 16th September 2020, conducted at Primary Health Care Center in Baghdad City, Iraq. Non probability (a purposive sample) the study sample consist of (150) women that attending Primary Health Care Center in Baghdad City, Iraq.

**Results:** indicates that there is significant relationship between women’s attitude and abstain of contraceptive methods as presented by significant correlation at p-value= 0.038.

**Conclusions:** there is significant relationship between women’s attitude and abstain of contraceptive methods related to Iraqi social habits that make men compel women to become pregnant and have a lot of children and there are other causes prevent women using contraceptive methods.

**Recommendation:** Designing programs to educate couples about family planning and using of contraceptive before marriage, Empowering the role of nurse in family planning to educate the women about contraceptive methods and how to use it and help them to choice the appropriate method, Activate the role of media in increase awareness of families in the Iraqi society about disadvantages of unplanned pregnancies and do not using contraceptive methods on women well-being and the luxury of community, and further studies to identifying the causes that make women do not use of contraceptive methods.

**Keywords:** Women, Attitudes, Contraceptives Methods, Abstain

**Introduction**

Family planning is defined as having the freedom and responsibility of all the couples and the individuals to decide the number of children they desire and having the knowledge, education and tools for this purpose. In other words, family planning is a preventive service that allows married couples achieving their desired number of children and deciding the spacing of pregnancies according to their economic opportunities and personal wishes, and to ensure that the births are at appropriate intervals for the mother and child health [1]. Over the past half century, an extensive literature has been accumulated on women’s attitudes and beliefs concerning contraception in general and specific methods. The extensive evidence for low- and middle-income countries (LMICs) falls into four main categories. The most common category comprises studies that examine attitudes towards and beliefs about contraception in general, with little or no distinction...
between specific methods. The emphasis in many papers is on negative perceptions, often labelled as myths and misinformation. Commonly reported themes include the belief that use of modern methods will cause long-term infertility, serious health damage, such as cancer and foetal abnormalities[2].

Low rates of unintended pregnancy are linked to access to contraceptive methods and legal access to abortion. Once women decide which tool to use, the efficacy of contraceptive methods is considered important. During contraceptive therapy, the efficacy of “typical use” rather than the “best usage” of contraceptive methods should be considered. The traditional use of contraceptive methods, however, may not be common knowledge among providers or women. Younger women have been shown to have less adherence to methods that require daily intake [3]. The most research regarding family planning methods is based on self-reported reasons for family planning non-use or discontinued use. Demo-graphic and health surveys (DHSs) are the dominant data source. The new study of reasons for non-use by married women who do not wish to become pregnant found that 20–33 percent of the 52 countries surveyed reported side effects or health concerns. This class of explanation has been the most widespread in 21 countries [4]. Moreover, the majority of women in a lot of countries who gave this reason for preventing birth control had already used a modern method. Fear of adverse effects, disapproval by couples, a limitation of option and knowledge and social disapproval give an explanation for unsatisfactory needs and low family planning use. Son preference has too been an issue in fertility especially in patrilineal societies, and this persists even with improvement in women’s levels of education social and economic-economic development. In addition, cultural differences have been highlighted, in particular traditional expectations and desires for more children and lines, as affecting family planning [5]. Important barriers to contraceptive methods use are myths and misunderstandings towards modern methods, for instance, in both developed and developing countries, many women misunderstand the use of oral contraceptives as being more dangerous than pregnancy [6].

**Methodology**

The study was started at 16th September 2019 to 16th September 2020, and the objectives were explained to the study sample by the researcher, the women’s verbal consent has been taken and the answering of questions have been done by using the self-administrative methods. In this study there is (150) women who were attending Primary Health Care Center in Baghdad City, Iraq. Which were selected according to inclusion criteria that are the females in reproductive age who attending Primary Health Care Center in Baghdad City, married women, and have children. The questionnaire consists of (3) parts, the first consists of Socio-Demographic Characteristics include: (age, level of education of women and their husband, occupation of woman and their husband, residency, socio economic status). The second consists of Reproductive History and includes (age at menarche, age at marriage, marriage duration, women parity, general information about children (gender, age, mode of delivery, and mode of feeding), age of mother at the primiparity and types of contraceptive methods that used and their duration. The third consists of Question about women attitudes is consist of (51) items which are divided in to three main domains first one is (effective domain) consist of (12) items, the second one is (behavioral domain) consist of (17) items, and the last one is (cognitive domain) consist of (22) items. participant answering the questionnaire to Evaluate their Attitudes about using of Contraceptive Methods. Some of questions had a positive Attitude and the other a negative Attitude, women that participate in this study were answering the questions by Owen selves and express their opinion about using of Contraceptive Methods without any disruption or direction from the researcher, so all the Attitudes that appear in the results it is express the Attitudes of Iraqi women about using of Contraceptive Methods.
## Results

### Table (1): Distribution of Women’s Socio-demographic Characteristics (SDCv.)

<table>
<thead>
<tr>
<th>SDCv.</th>
<th>Classes</th>
<th>Wife</th>
<th></th>
<th>Husband</th>
<th></th>
<th>C.S. (*) [P-value]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women's age group Yrs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 – 24 years</td>
<td>24</td>
<td>16%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 – 34 years</td>
<td>67</td>
<td>44.7%</td>
<td></td>
<td></td>
<td>P=0.042 (S)</td>
</tr>
<tr>
<td></td>
<td>35 – 44 years</td>
<td>44</td>
<td>29.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45 ≤ years</td>
<td>15</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average mean =</td>
<td>31±7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education:</td>
<td>Unable to read &amp; write</td>
<td>3</td>
<td>2%</td>
<td>3</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>25</td>
<td>16.7%</td>
<td>25</td>
<td>16.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intermediate school</td>
<td>30</td>
<td>20%</td>
<td>30</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>25</td>
<td>16.7%</td>
<td>14</td>
<td>9.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diploma (Institute)</td>
<td>29</td>
<td>19.3%</td>
<td>35</td>
<td>23.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bachelor (College)</td>
<td>30</td>
<td>20%</td>
<td>40</td>
<td>26.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Postgraduate</td>
<td>8</td>
<td>5.3%</td>
<td>3</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>Housewife / Jobless</td>
<td>81</td>
<td>54%</td>
<td>4</td>
<td>2.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee</td>
<td>68</td>
<td>45.3%</td>
<td>74</td>
<td>49.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Free Job</td>
<td>1</td>
<td>0.7%</td>
<td>68</td>
<td>45.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retired</td>
<td>0</td>
<td>0%</td>
<td>4</td>
<td>2.7%</td>
<td></td>
</tr>
<tr>
<td>Residency (both)</td>
<td>Urban</td>
<td>142</td>
<td>94.7%</td>
<td>-</td>
<td>-</td>
<td>P=0.375 (NS)</td>
</tr>
<tr>
<td></td>
<td>Sub-urban</td>
<td>8</td>
<td>5.3%</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>0</td>
<td>0%</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Monthly income (both) (Iraqi Dinars):</td>
<td>&lt; 300,000</td>
<td>26</td>
<td>17.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300,000 – 600,000</td>
<td>48</td>
<td>32%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>601,000 – 900,000</td>
<td>26</td>
<td>17.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>901,000 – 1,200,000</td>
<td>29</td>
<td>19.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,201,000 – 1,500,000</td>
<td>15</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,501,000 ≤</td>
<td>6</td>
<td>4%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (2): Correlation between Women’s Attitudes and Abstain (Used/Unused) Contraceptive Methods (N=150)

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Attitude</th>
<th>Abstain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitude</td>
<td>Pearson correlation 1 0.169</td>
<td>Sig.(2-taild) -- 0.038</td>
</tr>
<tr>
<td>Abstain</td>
<td>Pearson correlation 0.169 1</td>
<td>Sig.(2-taild) 0.038 --</td>
</tr>
</tbody>
</table>

Sig= significance

The table (2) indicates that there is significant relationship between women’s attitude and abstain of contraceptive methods as presented by significant correlation at p-value= 0.038.

Results and Discussion

Table (1) shows observed frequencies, and percentages of the studied socio-demographical characteristics variables (SDCV) which are distributed according to studied samples as well as that (44.7%) of women are with age group (25-34) years old and (29.3%) of them are with age group (35-44) years; the average mean for those women was refer to (31±7) year, (20%) among women who were graduated from college and intermediate school; while (19.3%) of them were having diploma degree. Educational level for women’s husbands, the highest percentages among them were indicated that their husbands are with bachelor degree (26.7%) and diploma degree (23.3%), half of women are housewives (54%) and (45.3%) are governmental employee, women’s husbands refers that (49.3%) of them are governmental employee and (45.3%) are Free job as presented the higher percentages among occupation for women’s husbands, majority of women are living in an urban area (94.7%) while only (5.3%) were resident in sub-urban areas, and (32%) of women are associated with monthly income of (300 000 - 600 000) Iraqi Dinars, (19.4%) of women are associated with moderate socio-economic status as reported that their income is (901,000 – 1,200,000) Iraqi dinar/ month, and (17.3%) of them are reported their monthly income is (<300,000 and 601,000-900,000) Iraqi Dinar.

Significant relationship between women’s attitude with regard to their age presented by significant difference between overall scale and age group at p-value ≤ 0.05. No significant relationship between women’s attitude with regard to their level of education, their occupation, their husband’s education, and their occupation at p-value ≤ 0.05. No significant relationship between women’s attitude with regard to their residency at p-value ≤ 0.05, no significant relationship between women’s attitude with regard to their socio-economic status at p-value ≤ 0.05.

The table (2) indicates that there is significant relationship between women’s attitude and abstain of contraceptive methods as presented by significant correlation at p-value= 0.038.

Table (3) shows that they got their first menstruation at age of (13 and 14) years old (27.3%), that half of women were marrying at the age (18-23) years old (50%), (20.7%) were marrying at age (24-29) years, that about third of women are reported (6-10) years as duration of their marriage (33.3%), and (29.3%) are reported that they married for (1-5) years ago, presented the descriptive characteristics of women’s children; the number of children is distributed among the women, all of them are reporting they have one child (N=150), 112 of them reported they have two children, 75 of them are reported they have three children, 44 of them are reported they have four children, 23 are having five, 8 are having six, only three having seven, and only one having eight children, and women are born their first child at age of (19 – 23) years with high percentage among women under the study (40%). That there is no significant relationship between women’s attitude
with regard to their age at menarche at p-value ≤ 0.05. Significant relationship between women’s attitude with regard to their age at marriage and their years of marriage indicated by significant difference between overall scale and age at p-value ≤ 0.05.

Conclusions

The study concluded the following:

1. The highest percentage of women were at age group (25-34) years old. Their educational level was intermediate school and bachelor and their husband had bachelor. Were housewives and their husband employee. They lived in an urban area with moderate income.

2. The highest percentage of women had menarche at age (13 and 14) years old. Half of them married at age (18-23) years old. Third of them had (6-10) years marriage duration. All of them have children and they have the first child at age (19-23) years old.

3. There is significant relationship between women’s attitude and abstain of contraceptive methods related to Iraqi social habits that make men compel women to become pregnant and have a lot of children and there are other causes prevent women using contraceptive methods.

Recommendations: Designing programs to educate couples about family planning and using of contraceptive before marriage, Empowering the role of nurse in family planning to initiate workshop to educate the women about contraceptive methods and how to use it and help them to choice the appropriate method, Activate the role of media in increase awareness of families in the Iraqi society about disadvantages of unplanned pregnancies and do not using contraceptive methods on women well-being and the luxury of community, and further studies to identifying the causes that make women do not use of contraceptive methods.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

Clinical and Histopathological Study of Diclofenac Sodium-Acetylsalicylic Acid Toxic Effect on Liver of Mice

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Abstract

Both over-the-counter (OTC) and prescription analgesics-antipyretics like ibuprofen which is nonsteroidal anti-inflammatory drugs (NSAIDs) have effective as well as safe use for long time. Reversible inhibition of cyclooxygenase enzymes is the mode of action of NSAIDs over all. Use of NSAID lead to drug adverse reactions such as cardiovascular and gastrointestinal bleeding also effects of renal. Drug–drug interactions (DDIs) within the NSAID and a concomitant medication cause adverse drug reaction to several cases. DDIs have been reported for example, when commonly used medications as well as some antihypertensive, alcohol, aspirin, antidepressants are coadministered with NSAIDs. Dependent on total drug exposure cause a continuous risk in that the potential for an ADR due to interactions pharmacologic nature. When assessing ADRs potential risk is important to consideration the use of NSAID dose and duration, also the comedication type or class administered. The sub acute toxicity were carried out on thirty mice divided to three equally groups (10 mice in sub group) which were dosed for two weeks daily as follows: group one (control) was givin distilled water, the group two was get diclofenac (voltaren) (100mg/kg B.W.) and group three was givin diclofenac (voltaren) with aspirin (100mg/kg B.W.) for each together. Clinical signs of toxicity appear as following: abdominal pain, nausea, dizziness, anorexia, ataxia, blueness of the chest area, shortness of breath and Paralysis of the posterior limbs and recumbence, finally the death of mouse. Serum liver enzyme (ALT and AST) were elevated in all groups (diclofenac and diclofenac with aspirin) as compare to control after two weeks. Considering histopathological studies of liver tissue after two weeks of daily treatment, which showed moderated mononuclear cells infiltration in portal area around blood vessels and bile duct for voltaren group and fatty vacuoles in the cytoplasm of hepatic cells, single cells necrosis and severe inflammatory cells infiltration in the capsular area for voltaren with aspirin group. We concluded that diclofenac and diclofenac with aspirin have toxic effect appear by clinical symptoms and damage to liver, furthermore, don’t administration of more than one NSAID at same time.

Key word: Nonsteroidal antiinflammatory drugs, voltaren Na, aspirin, toxicity, liver

Introduction

NSAIDs (Nonsteroidal anti-inflammatory drugs), are a class of medicine that lower fever and reduce pain, prevents blood clots, with high doses lead to decrease inflammation (1). The prominent members of this drugs group, aspirin; ibuprofen also naproxen, are mostly available in most countries as over the counter (2).

Adverse effects depend upon the drug specificity, but largely included an high risk of gastrointestinal ulcers with bleeds, also heart attack, as well as disease of kidney (3 and 4).

Achieve analgesia by any member of drug groups known as an analgesic or painkiller, pain relief, the work of analgesic drugs with different ways on the peripheral and central nervous systems. Anesthetics different from analgesic, which in some states eliminate, sensation completely, or mainly with temporarily affect. paracetamol one of analgesics (acetaminophen), the nonsteroidal anti-inflammatory drugs (NSAIDs) like salicylates, and opioid drugs, like morphine and oxycodone. Severity and response to other medication was determine that agent to choice when choosing analgesics; (WHO) the World Health Organization, pain ladder, its first step is specifies mild analgesics (5).
To choose analgesic is depend on pain type: The less effective analgesics are traditional to pain of neuropathic, also drugs classes which are not considered analgesics normally is often benefit in treatment, like anticonvulsants, also tricyclic antidepressants (6).

COX inhibit is mechanism of NSAIDs over all, which is an enzyme that change arachidonic acid to prostaglandins, by which pain mediating; fever; and inflammation (7).

Diclofenac is sold with the brand name (Voltaren) within others, is a NSAID (nonsteroidal anti-inflammatory drug) used for treatment of pain, also inflammatory diseases like gout, take with oral, injection, or skin applied, typically pain released get through half an hour and it lasts for about eight hours, in combination with misoprostol voltaren also available to decline problems of stomach (8).

The pain of abdominal, nausea, gastrointestinal bleeding, headache, dizziness, and swelling are the common side effect, otherwise serious side effects like stroke, disease of heart, kidney problems, and ulceration of stomach, it is contraindicated in third pregnancy trimester, also idicated through breastfeeding, it is work depend upon decreasing prostaglandin production (1). It lead to cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) blocks (8).

The widely used non-steroidal anti-inflammatory drugs used one is, probably is highly consumed pharmaceutical product mostly in the world. Aspirin, as a cardio-protective drug with an analgesic has a bigger importance recently. Otherwise, due to its adverse effects, the consumption of aspirin is depend on significant morbidity and mortality on many systems organ (9). Therapeutic use of aspirin for long-term is reported that accompanied to gastrointestinal ulcerations, hepatotoxicity, nephrotoxicity, also renal cell cancer occurrence (9 and10).

Acetylsalicylic acid (Aspirin), is a drug of salicylate, synthetic compound has analgesic, antipyretic, antiplatelet also anti-inflammatory properties (11). Children have little indications to use aspirin in case Kawasaki disease and Rheumatic fever (12).

NSAIDs protein binding were decreased when it administered with aspirin, although unknown of this interaction significance clinically, the free NSAID clearance was not changed (13).

Materials and Methods

Drugs:

1. Voltaren (Diclofenac)

Diclofenac (Voltaren) was tablet, 100mg/kg, Novartis, Switzerland.

2. Aspirin (acetylsalicylic acid)

Aspirin was tablet, 100mg/kg, Bayer, Germany.

Experiments:

Sub acute Toxicity Study:

Animals:

Thirty mice (Albino, Seprige Dawlly BO. CL) were obtained from the Serum and Vaccination Center. The mice 10 weeks old weighing 25-40 g were maintained under uniform environmental conditions. They were housed under controlled temperature 25 °C and exposed to light 12 hours per day before and during study. The mice were placed in cages (Opaque, plastic, measuring 29 x 15 x 12 cm). The mice were supplied with feed (Pellets form, commercial type) ad libitum. The animals were adapted for 2 weeks and allocated at the weighted groups.

The mice were split as three equal groups (10 mice to every group) which were to follows:

1- First control group treated with distilled water.
2- Second treated group by100mg/kg voltaren orally for 14 days.
3- Third group treated with 100mg/kg voltaren with 100mg/kg aspirin orally for 14 days.

Clinical signs

The signs was recorded to 14 days after administration of voltaren and voltaren with aspirin.
Parameter:

Blood serum sample:

Blood sample were collected from rabbits heart directly by (5cc syringe) in a dry, clean and sterile centrifuge tubes, and then left few minutes allowed to be clotted at room temperature before circulation by centrifuge at (3000) rpm for 20 minutes to separate the clear sera which were put in eppendorf tube by micropipette till performing the biochemical analysis (14).

Serum level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (AP).

Histopathology:

The mice treated for two weeks, doses found by previous studies as treated dose for mice, which used the 100mg/kg voltaren and 100mg/kg aspirin, liver was used as a sample in histopathological study after two weeks of experiment to each groups. The animal was killed by broken neck with scissor then the liver was obtained and put in plastic container contain formalin solution (10%) and stained with Haematoxylin and Eosin staini

Statistical Analysis

Statistical analysis of data was done by using SAS (Statistical Analysis System - version 9.1). ANOVA One-way and Little significant differences (LSD) test of post hoc were done to determine significant differences within means, significant statistically is consideration to P < 0.05 as described by (15).

Results

Subacute Toxicity:

Clinical signs

After administration of voltaren and compination of voltaren and aspirin drugs to the mice have many symptoms of toxicity appear in different degree according to the drug as following: Abdominal pain, Nausea, Dizziness, Anorexia, Ataxia, Blueness of the chest area, shortness of breath, Paralysis of the posterior limbs and recumbence, finally the death of mouse.

Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (AP):

The serum levels of liver enzyme (ALT, AST and ALP) were found to be relatively elevated in the animals received diclofenac and diclofenac with aspirin in all groups (table 1 and chart 1). Serum AST levels through treatment for two weeks showed significantly increase at (P<0.05) between diclofenac group and diclofenac with aspirin as compared to control group. The levels serum of Alanine aminotransferase (ALT) within two weeks of treatment showed significantly increase at (P<0.05) between diclofenac and control group and no significantly increase on (P<0.05) of diclofenac with aspirin as compared to group of control. The level of serum ALP after two weeks of treatment showed significant decrease at (P<0.05) between diclofenac group and control group, otherwise no significantly increase at (P<0.05) of diclofenac with aspirin as compared to control.

<table>
<thead>
<tr>
<th>Test Group</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.20±0.73c</td>
<td>13.40±1.07b</td>
<td>74.00±0.70a</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>39.20±2.35a</td>
<td>38.00±1.00a</td>
<td>47.80±3.73b</td>
</tr>
<tr>
<td>Diclofenac +Asprin</td>
<td>21.00±0.31b</td>
<td>14.60±0.92b</td>
<td>75.40±1.99a</td>
</tr>
</tbody>
</table>

The different letter with means in the same column are different significantly at (P<0.05)
Histopathological results

Results of liver histopathology in all groups after two weeks was obtained as following:

1. Group one (control group):

Histopathological sections of the liver tissue in control group after two weeks of the experiment (figures1), with no clear lesions.

(Figure 1): Histopathological section of the liver tissue to mouse treated with distilled water shows: no lesions clear (H & E stain 400X)
2. Group two (diclofenac group):

The lesions after two weeks of the experiment in liver tissue (figure 2) were shows moderated mononuclear cells infiltration in portal area around blood vessels and bile duct.

(Figure 2): Histopathological section of the liver tissue of mouse in diclofenac group after 2 weeks of the experiment shows moderated mononuclear cells infiltration in portal area around blood vessels and bile duct (H and E stain 400X).

3. Group three (diclofenac with aspirin group):

The lesions after two weeks of the experiment in liver tissue (figure 3) were shows fatty vacuoles in the cytoplasm of hepatic cells, single cells necrosis and severe inflammatory cells infiltration in the capsular area.

(Figure 3): Histopathological section of the liver tissue of mouse treated with aspirin and diclofenac shows fatty vacuoles in the cytoplasm of hepatic cells, single cells necrosis and severe inflammatory cells infiltration in the capsular area (H & E stain 400X).
Discussion

Diclofenac sodium symptoms include: Diarrhea, drowsiness, headache, dizziness, movement problems, nausea and vomiting (bloody sometimes), numbness and tingling, blurred vision, ringing in the ears, stomach pain (bleeding in stomach and intestines possibility), unsteadiness, rash, urination problems (no urine output to little), edema (body or legs swelling), wheezing (16 and 17) and this agreement with our present study which found some of this common symptoms.

Hepatotoxicity can be viewed as a result to an adverse interaction between two xenobiotic systems within the liver. Drug metabolizing enzymes have evolved as a result of relentless animal–plant warfare for a billion years.

Alternatively, an anti-inflammatory (also immune regulatory) property of IL-10, may be important in drug-induced liver injury (18) as proven by IL-10 knockout mice susceptibility to acetaminophen-induced hepatotoxicity. For that, the relation of cytokine polymorphisms with diclofenac-induced hepatotoxicity proven by in this study probably independent upon immune mechanisms. The liver’s ability to produce anti-inflammatory cytokines like IL-10 could, to an extent, be responsible for the low frequency of clinically significant hepatotoxicity (19).

Significant changes to the parameters of biochemical used for toxicity indicators. The levels of ALT, AST and AP in the blood normally are low concentration, those enzymes are considered as biomarkers of hepatic affection and elevated of these enzymes due to a major permeability or cell rupture (20).

Drug-induced liver injuries are classified as ‘hepatocellular’ (cytolytic), with an elevation of alanine transaminase (ALT) predominantly implying hepatocyte necrosis; ‘cholestatic’, with an elevation of alkaline phosphatase (ALP) predominantly, and ‘mixed’ pattern based on the ratio of ALT/ALP activity (19).

Liver transaminases (ALT with ALP) are benifit biomarkers of liver injury in a patient has some degree of healthy liver function, since ALP can be increased in other affecting organs diseases, ALT is more considered specific for hepatotoxicity (21).

Oxidative stress which caused by nitroxide and quinone imine-related redox cycling or putative diclofenac cation radicals and mitochondrial injury (opening of the permeability transition pore and protonophoretic activity) in combination or alone have been appeared in toxicity of diclofenac. For some cases, from a number of experiments demonstrating T cell sensitization and immune mediated liver injury is involved, inferred from unintended re-challenge data (21).

NSAID (non-steroidal anti-inflammatory drug) like diclofenac, using as antipyretic agent and an analgesic. Liver toxicity with diclofenac is lethal complication when it is not common with NSAIDs (22).

Hepatic degenerative changes by diclofenac which cause cellular damages (23). In this study, treated with diclofenac sodium lead to a significantly increased in ALT, AST, MDA,NO, and Caspase-expression accompanied by significant decline in total protein and albumin, also these results were accompanied by histopathological section and agree with the research (24) who reported that, increase of ALT, AST and ALT/AST, also inhibition of albumin and total protein are coinciding with the severity of damages of liver cell because aminotransferases are intracellular enzymes furthermore, rupture of hepatic cell due to circulation escaping of these enzymes ad this agreement with our study results. Damage of liver has been accompanied with induction of the pro-oxidant enzyme iNOS (25) producing NO. histopathological results of our study similar to those studied by (24) who evoked changes in histological section of liver. Diclofenac sodium appeared to cause hepatic damage by inducing alteration of biomarkers to limit the liver. One of the most sensitive and enthusiastic signs of liver cell damage is the entry of intracellular impulses, for example, transaminases and serum acid neutralizer phosphatase in the cycle (26).

(27) who found that histopathological study explained seriousness expanded of sores with expanded medication dosage, vacuolation with direct diffuse degeneration, peri-acinar rot with mellow to direct invasion of gateway zones and mononuclear cells in hepatic tissues analyzed of rats administrated diclofenac sodium for 14 days at doses of 100 and 150 mg/kg and this agreement with present study.
was found in histopathologically slides treated by aspirin explained that found a mild to moderate portal inflammation, focal cellular swelling, micro vesicular steatosis with cytolysis and this agreement with present study.

Mitochondrial adenosine triphosphate synthesis is impairs by aspirin, by direct cytotoxic effect or active metabolites leading to permeability elevated of mitochondria, oxygen reactive species overproduction, mitochondrial swelling, nicotinamide adenine dinucleotide phosphate oxidation and protein tiolls which can cause minimal interstitial inflammation with congestion to kidney tissue and hepatic injury. During intake of acute high-dose aspirin have been reported that liver function test abnormalities were reversible; although, during chronic low dose aspirin administration there is no clear data have been reported.

Aspirin lower concentration lead to peroxidation which causes impairment in human erythrocytes defense systems antioxidant was contraindicated used diclofenac with aspirin because possibility for drug interaction and this was clear from our present study with liver section for administrated diclofenac with aspirin together and this agreement with or hisotopathological result in present study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Dietary Habits of Iraqi Women with Breast Cancer at Oncology Hospitals in Baghdad City: Comparative Study

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Abstract

Background: Dietary pattern explains the overall diet; the food, food groups and nutrients included; their variety and combination. Breast cancer is the most prevalent cancer among women worldwide. Many risk factors for breast cancer contribute to personal habits, such as diet and exercise. Certain risk factors linked to lifestyle include decisions to have children and to take medicines containing hormones.

Objectives of the study: To assess women’s dietary Habits, Foods Choices, and to find out the relation between demographic characteristics and breast cancer.

Methodology: A descriptive and analytic study, non-probability (a purposive sample) consist of (100) women was carried out at three Oncology Hospitals in Baghdad City, Iraq. The study group consist of (100) women divided in two groups the first one include (50) women as a (study group) that attending An oncology Hospitals and the second include (50) women as a (control group) that attending the Primary Health Care Centers.

Results: Women in both groups are consuming red meat with low frequency and amount than normal consumption. More than half of women with breast cancer are eating red meat one time per week (54%) while those without breast cancer are eating red meat two times per week (50%).

Conclusion: Women in both groups are consuming red meat with low frequency and amount than normal consumption.

Recommendation: Avoid eating canned and processed red meat with preservatives and replaced it with fresh well cooked meat. Community orientation towards a healthy, balanced nutrition that contains the basic elements that the body needs without focusing on a specific food. Activation the role of media in increase the awareness of women in the community about healthy diet. Instruct women with breast cancer to follow a healthy diet guideline and avoid eating canned and processed red meat with preservatives during their treatment courses.

Keywords: Dietary Pattern, Breast Cancer, Oncology Hospitals, Comparative Study

Introduction

Dietary pattern explains the overall diet; the food, food groups and nutrients included; their variety and combination; and the amount and quantity with which they are typically consumed. Among the most common methods for determining dietary patterns are a priori numerical indexes, which measure adherence to a dietary pattern that has been predefined on the basis of previous scientific evidence. Numerous indices also define variants of the same dietary pattern (e.g. Mediterranean diet score) or use different scores and weighting systems, such as population-specific diets versus set intake reductions for prescribed intakes (e.g. Alternative Healthy Eating Index).

Another method is to empirically derive common patterns of foods that appear to be processed together
using principal component analysis or that explain the greatest variability in intermediate outcomes[1]. Many risk factors for breast cancer contribute to personal habits, such as diet and exercise. Certain risk factors linked to lifestyle include decisions to have children and to take medicines containing hormones[2].

No single food or diet can prevent or cause breast cancer, but while living with the disease, a person’s dietary choices can make a difference to their risk of developing breast cancer or their overall wellbeing. Breast cancer has many common factors and is a complex disease. Some of these variables are not within a person’s control, including age, family history, genetics and gender. A person can regulate other factors, such as smoking, levels of physical activity, body weight, and diet, though. Some researchers indicated that dietary factors could account for (30–40%) of all cancers[3].

Breast cancer is the most prevalent cancer among women worldwide. Humans have long known about breast cancer. The Edwin Smith Surgical Papyrus for example explains breast cancer cases. This medical text is from 3000–2,500 B.C. And probably due to Imhotep (Egyptian doctor-architect), offers authentic breast cancer accounts[4]. Food frequency intake consists of a finite list of foods and beverages with response categories that represent the normal level of consumption over the requested time period. The normal serving size can be ordered separately for each food and beverage.

To order to determine the overall diet, the amount of foods and beverages usually varies from (80 to 120). Alternatively, portion size can be paired with frequency information by asking respondents to convert the usual amount of consumption to the number of specified units (e.g. how often do you consume 1/2 cup of rice?). Many questionnaires contain portion size photographs in an attempt to improve reporting accuracy. Its objective is to obtain information on the frequency and, in some cases, the portion size of food and drink consumption over a specified period of time, usually the last month or year[5]. Dietary habits and decisions play a key role in human health. Dietary habits are common decisions made by individuals or groups of people as to what food they consume. Proper dietary choices include the use of minerals, vitamins, proteins, carbohydrates and fats[6].

Evidence has indicated that a healthy diet is associated with lower recurrence of breast cancer and increased overall mortality. Current food guidelines for cancer survivors have emphasized a plant-based diet rich in fruit and vegetables and low in red and processed meat[7]. Highly motivated individuals who maintain large reductions in their very long-term meat intake (> 3 servings per week) may gain benefits.

For people interested in reducing meat consumption (whether to improve health or mitigate the environmental effects of meat production), certainly nothing in this guideline argues against that lifestyle change[8]. Consuming meat can increase breast cancer risk. Possible causes include increased fat consumption, exposure to chemicals produced at high temperatures when cooking meat and exposure to meat hormones[9]. Some studies, however, suggest that women who eat a lot of processed meat (such as sausage and bacon) may be at increased risk of breast cancer[10].

**Methodology**

The data was collected through the use of a questionnaire. After getting the official approval from previous mentioned hospitals and primary health care centers, the sample was include (100) women divided in two groups the first one (Study Group) is consist of (50) women that with breast cancer that attending Oncology Hospitals and the second one (Control Group) consist of (50) women without breast cancer that attending Primary Health Care Centers in Baghdad City in the two directors AL-Karch and AL-Rasafa. For the purpose of dietary pattern assessment there are four methods including (Anthropometric measurement, Biochemical/biophysical methods, Clinical methods and Dietary assessment) in this study using the last one (Dietary assessment). The measurement of food and fluid intake is an essential part of the diet assessment. It provides information on dietary quality and quantity, food allergies, changes in appetite and explanations and sensitivity, for insufficient intake of food during or after illness. In this method the researcher collects the information from the participants depend on the recording or memory of the participant, so in this study the researcher depend on the memory of the women to collect the data about the dietary pattern because the data was collected about the dietary pattern before the
incidence of breast cancer and then compared with the dietary pattern of women without breast cancer. The time consumed for filling the questionnaire is 15-30 minutes.

**Results and Findings**

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</tr>
<tr>
<td></td>
<td>20 – 29 years</td>
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<tr>
<td></td>
<td>30 – 39 years</td>
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</tr>
<tr>
<td></td>
<td>40 – 49 years</td>
<td>6 18 9</td>
</tr>
<tr>
<td></td>
<td>50 – 59 years</td>
<td>19 50 25</td>
</tr>
<tr>
<td></td>
<td>60 ≤ years</td>
<td>3 12 6</td>
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<td>64 32</td>
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<tr>
<td>Housewife</td>
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<td>27 54</td>
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<tr>
<td>Retired</td>
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<td>5 5</td>
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<tr>
<td>Free Job</td>
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<td>40 64</td>
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<td>Rural</td>
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<tr>
<td>Sub-urban</td>
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<td>12 18</td>
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<td>Read and write</td>
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<td>24 12</td>
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<tr>
<td>Intermediate school graduate</td>
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<tr>
<td>Secondary school graduate</td>
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<td>5 10</td>
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<tr>
<td>Institute</td>
<td>18 9</td>
<td>5 18</td>
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<tr>
<td>Bachelor and above</td>
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<td>11 16</td>
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<tr>
<td>Extremely obese</td>
<td>4 2</td>
<td>1 4</td>
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<td>11 10</td>
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<tr>
<td>1,201,000 – 1,500,000</td>
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<tr>
<td>1,501,000 ≤</td>
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<tr>
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Table (2): Distribution of Women according to their Dietary Habits

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<tr>
<td></td>
<td>%</td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>Beef</td>
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<tr>
<td>Sheep</td>
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<tr>
<td>Both</td>
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<td>100</td>
</tr>
<tr>
<td>Eating red meat with:</td>
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<td></td>
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</tr>
<tr>
<td>Breakfast</td>
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<td>Lunch</td>
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<tr>
<td>Between meals</td>
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<td>2</td>
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<tr>
<td>More than meals</td>
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<tr>
<td>Eating meat at restaurant</td>
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<td>Preferred food</td>
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<tr>
<td>Red meat</td>
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<td>White meat</td>
<td>18</td>
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<tr>
<td>Vegetables</td>
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<td>More than one type</td>
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</tr>
<tr>
<td>Type of red meat</td>
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<tr>
<td>Frozen</td>
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<td>Processed</td>
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<td>6</td>
</tr>
<tr>
<td>Others</td>
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</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>50</td>
<td>100</td>
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</table>

f: Frequency, %: Percentage
Table (3): Evaluation of Red Meat Frequency Consumption per Week among Women

<table>
<thead>
<tr>
<th>Sig.</th>
<th>P-value</th>
<th>X2</th>
<th>Control group</th>
<th>Case group</th>
<th>Frequency Red meat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ass.</td>
<td>M</td>
<td>Ass.</td>
</tr>
<tr>
<td>N.S</td>
<td>0.954</td>
<td>0.681</td>
<td>Low</td>
<td>0.30</td>
<td>Low</td>
</tr>
<tr>
<td>H.S</td>
<td>0.001</td>
<td>11.735</td>
<td>Low</td>
<td>0.08</td>
<td>Low</td>
</tr>
<tr>
<td>H.S</td>
<td>0.001</td>
<td>50.639</td>
<td>Low</td>
<td>0.30</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.974</td>
<td>0.499</td>
<td>Low</td>
<td>0.38</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.552</td>
<td>0.335</td>
<td>Low</td>
<td>0.05</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.980</td>
<td>2.530</td>
<td>Low</td>
<td>0.43</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.694</td>
<td>3.875</td>
<td>Low</td>
<td>0.63</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.982</td>
<td>1.098</td>
<td>Low</td>
<td>0.45</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.312</td>
<td>7.095</td>
<td>Low</td>
<td>1.10</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.183</td>
<td>3.367</td>
<td>Low</td>
<td>0.55</td>
<td>Low</td>
</tr>
<tr>
<td>S</td>
<td>0.050</td>
<td>3.618</td>
<td>Low</td>
<td>0.18</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.837</td>
<td>0.043</td>
<td>Low</td>
<td>0.03</td>
<td>Low</td>
</tr>
</tbody>
</table>

M: Mean, Ass: Assessment, $X^2$: Chi-square, P: Probability, Sig: Significance

N.S: Not significant, S: Significant, H.S: High significant

Low = 0 – 1.33, Moderate = 1.34 – 2.67, High = 2.68 – 4

Table (4): Evaluation of Red Meat Amount Consumption per Week among Women

<table>
<thead>
<tr>
<th>Sig.</th>
<th>P-value</th>
<th>X2</th>
<th>Control group</th>
<th>Case group</th>
<th>Amount Red meat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ass.</td>
<td>M</td>
<td>Ass.</td>
</tr>
<tr>
<td>N.S</td>
<td>0.393</td>
<td>2.990</td>
<td>Low</td>
<td>0.30</td>
<td>Low</td>
</tr>
<tr>
<td>H.S</td>
<td>0.001</td>
<td>12.650</td>
<td>Low</td>
<td>0.08</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.777</td>
<td>1.778</td>
<td>Low</td>
<td>0.31</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.903</td>
<td>0.203</td>
<td>Low</td>
<td>0.38</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.629</td>
<td>0.203</td>
<td>Low</td>
<td>0.05</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.457</td>
<td>3.636</td>
<td>Low</td>
<td>0.43</td>
<td>Low</td>
</tr>
<tr>
<td>N.S</td>
<td>0.362</td>
<td>6.572</td>
<td>Low</td>
<td>0.55</td>
<td>Low</td>
</tr>
</tbody>
</table>
Table (4): Evaluation of Red Meat Amount Consumption per Week among Women

<table>
<thead>
<tr>
<th></th>
<th>M: Mean</th>
<th>Ass: Assessment</th>
<th>X²: Chi-square</th>
<th>P: Probability</th>
<th>Sig: Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shawarma (as)</td>
<td></td>
<td>Low</td>
<td></td>
<td>0.43</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>N.S</td>
<td>0.688</td>
<td>3.915</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>A piece of meat with soup</td>
<td>N.S</td>
<td>0.754</td>
<td>1.898</td>
<td>Low</td>
<td>0.74</td>
</tr>
<tr>
<td>Meat kebab</td>
<td>N.S</td>
<td>0.094</td>
<td>10.837</td>
<td>Low</td>
<td>0.76</td>
</tr>
<tr>
<td>Meat sausage</td>
<td>N.S</td>
<td>0.754</td>
<td>1.898</td>
<td>Low</td>
<td>0.02</td>
</tr>
<tr>
<td>Others</td>
<td>N.S</td>
<td>0.903</td>
<td>0.203</td>
<td>Low</td>
<td>0.02</td>
</tr>
</tbody>
</table>

M: Mean, Ass: Assessment, X²: Chi-square, P: Probability, Sig: Significance
N.S: Not significant, S: Significant, H.S: High significant
Low= 0 – 1, Moderate= 1.1 – 2, High= 2.1 – 3

Results and Discussion

The analysis of table (1) shows that the highest percentage regarding women’s age is refer to age (50 – 59 ) years among the women in the case study group (50%) and the women in the control group (38%). The occupational status reveals that more than half of women in both group; the case and control group are housewives (58%) and only (32%) among case group and (30%) among control group are governmental employee. Regarding residency, the women are reporting that they are resident in an urban areas (case group= 64% and control group= 80%). The educational level variable indicates that the highest percentage is referring that women are reading and writing among both groups; the case and control groups (24%). More than half of women in both groups are showing normal marital status that are married (case group=62% and control group= 66%). Regarding body mass indicators, the highest percentage is referring that women are overweight in both groups; the case and study (38% and 44%). The socioeconomic status is referring to moderate as presented by highest percentage of monthly income that is (601,000-900,000) Iraqi dinars among both groups (case group=50% and control group=30%).

The table (2) reveals that women are preferring sheep meat (study group=70% and control group= 58%). The women are preferred to eat red meat with lunch meal (study group=68% and control group=50%). (24%) of women with breast cancer are eating red meat at restaurant while (38%) of women without breast cancer are eating at restaurant. Those with breast cancer are preferred more than one type of red meat (56%) while those without are preferred the red meat (50%). They preferred the fresh meat (study= 82% and control= 66%).

The table (3) depicts the evaluation of frequency of red meat items consuming by women; the finding shows that women in both groups are consuming red meat low frequency than normal consumption as indicated by the low mean scores for all item, but the findings indicate that the boiled meat and Pasty have high significant among the items of red meat in addition to meat sausage that show significant also.

The table (4) depicts the evaluation of amount of red meat items consuming by women; the finding shows that women in both groups are consuming red meat with low amount than normal consumption as indicated by the low mean scores for all item, but the findings indicate that the boiled meat has high significant among the items of red meat consumption.

Conclusion

Women in both groups are consuming red meat with low frequency and amount than normal consumption.
**Recommendation:** Avoid eating canned and processed red meat with preservatives and replace it with fresh well-cooked meat. Community orientation towards a healthy, balanced nutrition that contains the basic elements that the body needs without focusing on a specific food. Activation the role of media in increase the awareness of women in the community about healthy diet. Instruct women with breast cancer to follow a healthy diet guideline and avoid eating canned and processed red meat with preservatives during their treatment courses. Presence of nutritional consultant in every oncology hospital to guiding all women with breast cancer about a healthy diet and finally more studies to identify the impact of nutrition upon the incidence of breast cancer in women.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**

1- Cespedes EM, Hu FB. Dietary patterns: from nutritional epidemiologic analysis to national guidelines.


Parents’ Knowledge Concerning School Phobia of their Children in Baghdad City, Iraq

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PhD, Ass. prof, psychiatric & Mental Health Nursing Department, college of nursing, Babylon University

Abstract

Background: Fear from school can also be described as any rejection by a child to join a school or to have difficult time attending the school for a complete day by a child. Aims of the study: To describe parents knowledge of their children with fear from school. Methodology: This study examined the analysis on the data collected by the Ibn Alhythm primary School all through from the middle of October till the end of January 2011 school year. The sample data took the form of surveys, questionnaires, and interviews by a researcher with parents who have been analyzed to investigate the associations among variables related to fear from school behaviour. “Ibn Alhythm primary School District’s Special Education Department collected data during the 2010-2011 school year. In hopes of identifying a representative sample of the school-refusing students in the district, the Special Education Department sent participation requests to a random sample of 40 of the 40 families described as having students who missed 18 or more days of the Ibn Alhythm primary School during the 2010-2011 school year. The information obtained did not include information from any students who were in Ibn Alhythm primary School through 1st grade or who missed fewer than 18 days of school, the information has been analyzed through data analysis. The results: The results of the study revealed that the educational program had a positive impact on the knowledge of family members. The results of the study have demonstrated that there is a correlation between the mothers’ knowledge and their demographic characteristics (age of parents, educational level, occupation, residential area). In general, knowledge of parents related to fear was low. However, the parents applied preventive measure towards their children with fear. A quasi-experimental study was conducted in the Ibn Alhythm primary school from the middle of October till the end of January 2011. Recommendation: Keep the sample group large. The author should get a fair representation of the population in doing so. Parents having a minimum level of experiences at school phobia, need a specific education program and training sessions. A specific education program can be designed and described to parent’s who have a minimum level of knowledge to enhance their level of knowledge.”

Keywords: Parents, Knowledge, child, Fear

Introduction

School fear can also be described as any child’s reluctance to go to school or having a child’s trouble attending classes for a whole day (1,2). According to (3,4) children missing long periods of school time, skipping classes, arriving late at school, missing sporadic school time periods, displaying severe morning misbehaviors in attempts to refuse school, attending school with great dread and somatic complaints. According to (5), school anxiety is present in around 5 per cent of children of school age. Left untreated, school phobia can lead to many dysfunctions in the long term. According to (4,5), school behavior fear is highly comorbid with various mental disorders such as segregation anxiety disorder (SAD), generalized anxiety disorder (GAD), oppositional defiant disorder (ODD), and depression. Most children who fear school actions have a variety of issues that internalize and outsource them. Problems of internalization include general and social anxiety, terror, weariness, sociality, and somatic complaints. Outsourcing issues include non-compliance with parent and teacher orders, disobedience and violence, running away from school or home, clinging and temper tantrums.
Popular elements among anxiety-based features like high school fear include a high degree of anxiety, a power struggle between students and either or both parents over the perceptions of helplessness of the students, inability to resist a powerful parent or parents, fear of failure to measure, thoughts that love is conditional on parental expectations, a propensity to ignore or avoid difficulty.

In order to treat school fear, it is important to have an understanding of the function of school refusal of a child. According to (8,9), children typically keep refusing school for one or more of the following functional conditions, including: to avoid school-based stimuli that give rise to a general sense of negative affectivity; to run away aversive school-based social and assessment situations.

Methodology

“The descriptive study included the review of data obtained during the (2010-2011) school year by Ibn Al-hythm Primary School. A representative sample of school-refusing students in their situation, the Department of Special Education sent requests for inclusion to a random sample of 40 out of 40 families identified as having children missing 18 or more days of Ibn Al-hythm primary school (10%) of the total number of days representing a full school year) in the (2010-2011) academic year. The data collection did not include information from any students who had been through 1st grade at Ibn Al-hythm primary school or who had missed less than 18 school days.

The instrument is composed of seventeen questions, four per state of maintenance. problem is rated on a scale of between 0 and 6, from never to ever. Once the scale is applied individually to children and parents, means are measured and ranked for each case. The highest-scoring condition is considered the primary variable preserving school refusal activity for a particular child.

The Questionnaire for the Parental Authority-Adapted Student Version (PAQ – ASV). (2) developed a PAQ to calculate three prototypes of parental authority for Baum rind (1971). Such three variants include authoritarianism, permissiveness and authoritarianism. The questionnaire consists of 30 items which yield permissive, authoritarian, and authoritative scores for both the mother and the father. Every object is rated on a scale of 1 to 5, ranging from sharp disagreement to strong agreement. This scale has been field-tested and found to be a valuable tool for the investigation of parental permissiveness, authoritarianism and authoritarianism correlates.

Results

Table- 1 - Characteristics of the Demographical data

<table>
<thead>
<tr>
<th>Demographical data</th>
<th>Group</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>Parent status</td>
<td>Single-parent</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Two-parent</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Socio-economic Status</td>
<td>Middle class</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Lower class</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Grade</td>
<td>2nd</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>5th</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>6th</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>
Table (1) indicates that a major percentage of male gender was (60%) while a high percentage of Single-parent status was (60%). However, the lowest percentage (40%) was two parents. Also, a majority percentage of 3rd Grade was (22.5%) regarding disability about 10 have speech and language problem and a high percentage of Medical illness was allergies (15%) while the lowest percentage (2.5%) was Head Lice.

Table (2) indicated the high percentage of All Functions Rated Infrequent or Never function of school phobia behaviour was (37.5%), but low percentage (2.5%) was Frequent Avoidance and Attention.

### Table -1 - Characteristics of the Demographical data

<table>
<thead>
<tr>
<th>Disability</th>
<th>Speech and Language</th>
<th>Specific Learning Disability</th>
<th>Emotional Disturbance</th>
<th>Gifted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medical illness</th>
<th>Allergies</th>
<th>Head Lice</th>
<th>Female Reproductive Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Table -2 -Function of Fear From School Behavior

<table>
<thead>
<tr>
<th>Function</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Functions Rated Infrequent or Never</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td>Frequent Tangible Reinforcement</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Frequent Attention and Tangible Reinforcement</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Frequent Avoidance</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Some Avoidance and Some Attention</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Frequent Avoidance and Attention</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Some Avoidance</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Some Attention and Some Tangible Reinforcement</td>
<td>1</td>
<td>2.5”</td>
</tr>
</tbody>
</table>
Table -3-“Function of School Refusal Behavior as Rated by the Parent (SRAS-Parent)”

<table>
<thead>
<tr>
<th>“Function”</th>
<th>F.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequent Tangible reinforcement</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td>All Functions Rated Infrequent or Never</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Frequent Avoidance And Tangible Reinforcement</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Frequent High Avoidance</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>Avoidance, Escape, Attention, And Tangible reinforcement</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>Some Attention</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Some Avoidance And Some Attention</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Avoidance And Escape</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Frequent Avoidance And Attention</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Frequent Avoidance, Attention, And Tangible reinforcement</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table(3) displays the major Percentage of a function of school phobia behaviour was (15) (37.5%). However, low percentage (2.5%) Frequent Avoidance, Attention, And Tangible reinforcement.

Table - 4- “Perception of Parent’s Parenting Style as Rated by the Student” (PAQ-Adapted-Student)

<table>
<thead>
<tr>
<th>“Parenting Style”</th>
<th>F.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authoritatives Style</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Authoritarian and Authoritative Style</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Authoritarian Style</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Not permissive or Authoritarian Style</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Not permissive or Authoritative Style</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Not permissive, Authoritarian, or Authoritative Style</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Not Authoritarian Style</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Not permissive, Style</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>High Authoritarian,and permissive Style</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>All styles Rated equally Style</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Permissive Style</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>


Table (4) demonstrates the high percentage of Adapted-Student was Authoritative (35%), but the low Perceptions (2.5%) was Permissive.

Table 5: Perception of Parenting Style as Rated by the Parent (PAQ-Adapted-Parent)

<table>
<thead>
<tr>
<th>Parenting Style</th>
<th>F.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authoritative Style</td>
<td>24</td>
<td>60.0</td>
</tr>
<tr>
<td>Authoritarian and Authoritative Style</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Permissive Style</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Authoritarian Style</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Permissive and Authoritative Style</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table (5) indicated the high percentage of perceptions of adapted-parent was authoritative (60%), also the low percentage (2.5%) was permissive.

Discussion

“"This current study is analyses simple data obtain from New Baghdad Area of Ibn Al-hythem primary School District in an attempt to propound and to answer four questions concerning the nature of school refusal in the Ibn Al-hythem primary School District. The study answered the following questions. Results of this study shown that the incident rate of school-refusing students in the Ibn Al-hythem primary School District was (14.75)% during the 2010-2011 school year. This rate, according to (8) is alarming because it is almost three times the rate reported thru (9); they described at that time that the prevalence rate of school refusal in school-aged children was (5%). These results suggest that school refusal behaviour is a very serious problem that needs to be addressed by the Ibn Al-hythem primary School District. High proportions of school refusal behaviour may contributed to improved drop-out rates and the reduced probability of students in the Ibn Al-hythem primary School District attending college (10). As a outcome, this number may be underrepresented in the sample. Beside, some students were might have falsely informed having a medical illness to make an excuse for their extreme absenteeism.

Regards to race, 92.5% of the sample was composed of, and the remaining (7.5)% of students were equal among races (10). While these results are not consistent with outcomes that recommend school refusal behaviour is equal among races, these results are fairly representative of the general student population in the Ibn Al-hythem primary School District more of the students in the simple data sample, who were distributed evenly across grades (3), missed (18-23) days of school. No significant differences at school refusal behavior were found between the ten different grades in the study. Research suggests that school refusal behaviour is most prevalent during transition years in school (3). Findings of current study shown most students in the sample were from single-parent homes (60%). According to the study of (6), family and marital conflict can trigger school refusal behaviour. The students were had two-parent households may have skilled just as much, if not more, family and marital conflict as those students, who survived in single-parent households. Also, it is not pure if the parents of students living in single-parent households lived with a paramour or significant other.

Around 40 students recorded a missed school in the study to seek measurable strengths (35 per cent). The pursuit of tangible reinforcements occurred most
often of the four possible functions of school refusal behaviour. Convincingly, most parents in the sample indicated that their children missed school primarily for tangible reinforcements. Such findings indicate that both students and parents have the same perceptions of school rejection behavior’s primary function. This is a issue as evidence suggests that this school refuse feature appears to be more persistent than the others, and is generally correlated with severe family conflict or issue family dynamics (7).

Worthy of note is the fact that 37.5 per cent of the student sample and 30% of the parent sample approved both things on the SRAS as uncommon or never occurring. These results indicate that viewpoint in reaction may have influenced the majority of students (35 percent) and parents (60 percent) viewed the household’s parenting style as the Authoritative Type. This model of parenting style is closely associated with middle-class ideals and involves children as authoritative parents guide their children by two-way choices in the decision-making process. These results are not consistent with the research which indicates that authoritative parenting generally predicts positive adjustment among children and adolescents (9).

One possible explanation for these results may be attributed to response on the PAQ. Students as well as parents may have supported the responses they felt would be more beneficial. As a result, these study results might not be an accurate representation of the true perceptions of parenting style of the students and parents. First, 15 percent of the student sample and 27.5 percent of the parent sample considered an Authoritarian and Authoritative parenting style in their children. Authoritarian style of parenting has been observed more often in lower class families than in middle class families. from oppressive backgrounds tend to be overly self-conscious and rebelling. This rebellious streak also contributes to drug abuse. Children from authoritarian upbringings, too, tend to be distrustful, more hostile and more resentful towards their parents, and are rarely high attaining children (9,10).

Surprisingly, only four students 10% perceived their parents as being solely in an Authoritarian parenting style, and no students perceived their parenting style as permissive. Similarly, 7.5 per cent of the parent sample perceived their style of parenting as Authoritarian. Lastly, only 2.5% of the parent sample endorsed a permissive style of parenting. Findings indicate that the same types of parenting styles were commonly regarded by both students and parents in their homes. Limitations of the study are the small sample size acquired from the shelf data from a single school district that limits the extent to which generalizations can be made to the population.”

Conclusions

A thorough investigation of the child be done to rule out any medical concerns and if necessary professional counseling should be required to help the child.

Recommendations:

1. Keep large size of sample. In so doing the researcher will have a good representation of the population.

2. Parents having the minimum level of practices at fear from school need a specific education and training programs.

3. A specific a education programs can be designed and presented to parent’s who have a minimum level of knowledge to enhance their levels of knowledge.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Maternal Anxiety Levels with Newborns in the Neonatal Intensive Care Unit at Hilla Maternity and Childbirth Hospital in AL-Hilla City

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Abstract

Aims: To comparing maternal anxiety levels for infants in the NICU and mother in the postnatal care-service (PCS) for stable newborns. Methodology This descriptive study was carried out in Al-Hilla Children’s and Birth Hospital, Al-Hilla City. The 1st 200 families, counting pregnant mother in personal care services (n=100) and pregnant women in neonatal intensive care unit (n=100), backed up by participant as of May, 2018. As data gathering tools, including the STAI TX-1-STAI TX-2 inventory, questionnaires were used to detect the characteristic of mother and newborn. Results Characteristic rates of anxiety were not substantially different between NICU neonate mothers and PCS neonate mothers (t = 0.588, p = 0.557), while status level of anxiety varied greatly among the 2 classes (t = -5.109, p = < 0.001). Motherland anxiety level were determined to be higher for mother whose children in the NICU compared with those of mother whose children were on personal care-services. Conclusion It can boost anxiety and lead to mothers being a mother to a newborn sick. During this difficult time, nursing care may rise mothers’ ability to deal with the sick stresses of the newborn

Keywords: Maternal, Anxiety, Newborn

Introduction

NICU conceded as a frightening place for parent because it’s loud, humid and crowded; however, sophisticated surgical technology and complicated medical terminology are obstacles for parent and newborn (1). Parent, particularly newborn mother living at NICU, may have a psychological problem with having a sick child and may think of losing their child and failing. Achieving conventional positions in parenting. (2-4) Lack of an appropriate environment in which mother can meet their children’s physiological needs (nutrition, drinking, sleep, etc.), In addition to their child’s illness, lack of detail on therapies and medical operations, poor communication with healthcare workers and not taking part in childcare, poor social support to high levels of anxiety (5,6)

Techniques

This descriptive study was carried out in hospitals of Maternity and Childbirth situated in Babylon, Iraq. Energy analysis was used to decide the size of sample; as a result of an energy investigation established on an earlier Yurdakul et al study. For each group of 95 mothers, this study identified 85 per cent of power (7). By assessing the 1st 100 mothers inside every group (PCS or NICU) who decided to take part in this research, this study reached 86 percent energy.

Tools of data-collection

Tool for data-collection involved a concise questionnaire model developed by Literature 5-7-10 and the General Status Anxiety Inventory (STAI TX 1 – STAI TX 2) to assess level of maternal anxiety. Hospitals had obtained the requisite permits for this report. Participants received informed consent. The descriptive questionnaire helps to evaluate mothers’ social and ethnic characteristics, as well as certain newborn characteristics. The additional descriptive questionnaire focused on maternal beliefs about the NICU and their child’s condition within the NICU. General Status Anxiety Inventory (STAI TX 1 –STAI TX 2)
Case Anxiety Inventory: such a measure defines whether a person feels in a given location or situation.

The Inventory of Trait Anxiety: such a standard analytical methods how a person feels regardless of facts of the case.

A type Likert scale, established through Spielberger et al. It is composed of forty items: twenty items measured by state anxiety, and twenty items measured by anxiety apps. On each measure the cumulative score obtained was measured separately. To this value was added a predefined and unfixed number; this number was fifty for country anxiety, and 35 for anxiety attribute. The consequence was the individual degree of anxiety. (11) Orener and Lumbeti adapted that meter to Arabic in 1983. (12).

Analysis and Data collection

Hospital and unit where this research was carried out obtained written permission and approval. Samples were knowledgeable of the purpose of the study and their oral informed consent was attained prior to the beginning of the study. During personal interviews, the researchers were given instruments for the data collection to participants. IBM SPSS 2 assessed the data obtained from the study.

Results

Table 1: Socio demographical Characteristics of sample.

There’s a significant dissimilarity in the State anxiety rating level for 2 classes of mothers. State anxiety level were determined to be higher for mother whose children were in neonatal natal intensive care unit related to those were in PCS.

<table>
<thead>
<tr>
<th>Table 2: State Trait Anxiety Levels of Mothers.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers with newborns in a NICU</strong></td>
</tr>
<tr>
<td><strong>Mean±SD</strong></td>
</tr>
<tr>
<td><strong>State Anxiety Levels</strong></td>
</tr>
<tr>
<td><strong>Trait Anxiety Levels</strong></td>
</tr>
</tbody>
</table>

*p<0.05

There’s no significant association among socio demographical-characteristic (age, level of educational, employment status and level of income), trend and state level of anxiety between mother whose children were in NICU and PCS. There was an important link between the involvement of help networks in the NICU and the state rates of mothers with children. By many mothers who had newborn babies in the NICU there have been significantly higher levels of public anxiety reported by those without a support system than those with the aid systems.
Table 3: Distribution of a “state-trait level of anxiety” by mother variables.

<table>
<thead>
<tr>
<th></th>
<th>Mother with newborn in a NICU</th>
<th>Mean ± SD</th>
<th>Mother with newborn in a PCS</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STAI TX-1</td>
<td>STAI TX-2</td>
<td>STAI TX-1</td>
<td>STAI TX-2</td>
</tr>
<tr>
<td>Age</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.102</td>
<td>-0.110</td>
<td>-0.132</td>
<td>-0.026</td>
</tr>
<tr>
<td></td>
<td>0.312</td>
<td>0.275</td>
<td>0.191</td>
<td>0.799</td>
</tr>
<tr>
<td><strong>Level of Educational</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary School</td>
<td>48.40±6.49</td>
<td>40.03±6.20</td>
<td>43.96±6.91</td>
<td>40.74±5.32</td>
</tr>
<tr>
<td>High School</td>
<td>46.37±7.24</td>
<td>38.03±5.46</td>
<td>42.72±6.44</td>
<td>39.73±6.16</td>
</tr>
<tr>
<td>University</td>
<td>50.28±5.49</td>
<td>39.57±7.24</td>
<td>40.28±4.46</td>
<td>35.71±1.70</td>
</tr>
<tr>
<td>F=1.009, p=.369</td>
<td></td>
<td></td>
<td>x²KW=0.961, p=0.399</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>48.75±6.69</td>
<td>39.04±5.05</td>
<td>42.28±5.39</td>
<td>39.12±4.54</td>
</tr>
<tr>
<td>Not Working</td>
<td>48.16±6.58</td>
<td>40.32±5.55</td>
<td>43.58±6.87</td>
<td>39.73±6.12</td>
</tr>
<tr>
<td>t=0.242, p=0.809</td>
<td></td>
<td></td>
<td>U=481.50, p=0.388</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>U=389.50, p=0.784</td>
<td></td>
</tr>
<tr>
<td><strong>Level of Income</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad</td>
<td>46.80±7.81</td>
<td>41.37±2.92</td>
<td>43.16±5.11</td>
<td>37.60±6.46</td>
</tr>
<tr>
<td>Moderate</td>
<td>49.09±6.73</td>
<td>39.62±5.82</td>
<td>44.02±6.93</td>
<td>40.00±6.31</td>
</tr>
<tr>
<td>Good</td>
<td>45.43±4.03</td>
<td>41.67±4.54</td>
<td>41.37±6.03</td>
<td>39.56±4.17</td>
</tr>
<tr>
<td>F=2.580, p=0.081</td>
<td></td>
<td></td>
<td>x²KW=0.740, p=0.691</td>
<td></td>
</tr>
<tr>
<td>Supporting Systems Presence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45.60±5.68</td>
<td>40.03±5.46</td>
<td>43.16±6.42</td>
<td>39.07±3.79</td>
</tr>
<tr>
<td>No</td>
<td>49.60±6.61</td>
<td>39.44±3.84</td>
<td>44.22±7.58</td>
<td>38.01±2.90</td>
</tr>
<tr>
<td>t=3.027, p=0.030</td>
<td></td>
<td></td>
<td>U=821.00, p=0.462</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>U=885.00, p=0.827</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>U=1062.00, p=0.584</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05

There’s no significant relation seen amongst baby’s gender and birth weight with the NICU mothers’ state-trait anxiety rates (Table-IV). There’s also no correlation between both the NICU mothers’ trait anxiety levels and the duration of hospitalization, The status of the child’s situation and the participation status in child care (p>0.05); there was a positive correlation seen concerning mothers’ state anxiety levels and those character traits.
Table 5: “State-Trait Anxiety Levels” according to the characteristic of the child.

<table>
<thead>
<tr>
<th>Birth Weight</th>
<th>≤2500 gram (n=35)</th>
<th>&gt;2500 gram (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>STAIX TX-1</td>
<td>48.61±5.70</td>
<td>48.31±7.21</td>
</tr>
<tr>
<td>STAIX TX-2</td>
<td>39.89±4.78</td>
<td>40.30±5.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>STAIX TX-1</td>
<td>47.97±7.13</td>
<td>48.71±6.42</td>
</tr>
<tr>
<td>STAIX TX-2</td>
<td>39.51±6.45</td>
<td>40.59±4.74</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Getting information about infant</th>
<th>Yes</th>
<th>Partially</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>STAIX TX-1</td>
<td>47.64±6.64</td>
<td>48.44±7.00</td>
<td>52.37±5.37</td>
</tr>
<tr>
<td>STAIX TX-2</td>
<td>39.55±3.41</td>
<td>40.21±6.01</td>
<td>40.81±5.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contribution in the care of the baby</th>
<th>Yes</th>
<th>Partially</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>STAIX TX-1</td>
<td>45.47±6.42</td>
<td>46.44±6.14</td>
<td>50.35±7.79</td>
</tr>
<tr>
<td>STAIX TX-2</td>
<td>40.42±5.53</td>
<td>40.50±5.77</td>
<td>39.09±5.13</td>
</tr>
</tbody>
</table>

*p<0.05

**Discussion**

Going to maternity is a difficult task and raising a child with a health condition can be more complicated and challenging.\(^{(13)}\) Levels of anxiety in mothers whose babies were in the intensive care unit were higher related to mothers with PCS, while anxiety rates in features were no different in the two classifications (second table). There have also been other studies which have close findings to our research.\(^{5.7-9.14}\)

During this period, mothers need psychological and social help to support the emotional equilibrium of mothers and reduce negative feelings that may clue to adverse health results.\(^{(15)}\) In our research, there was no link among the typical PCS status and anxiety levels and the availability of support systems; further, the level of state concern was low for mothers with strong support systems (Table II). There have been studies which have defended our results.\(^{7, 9, 15, 16}\) Comparing mothers with children in NICU and PCS, there was no significant relationship between the social, obstetric and demographic characteristics of mothers (age, educational status, income level) and anxiety levels (Table 3). Similar to other tests,\(^{7,8}\) Parents whose children have been admitted into NICU need details, especially from nurses, and communicate easily with nurses.

With the current study, the level of state anxiety was higher for mothers whose children were in the intensive care unit and who did not get sufficient information about their child care compared to the aspiring mothers (Table 4). Several studies have shown that having adequate information about an infant and successful interactions with health providers has led to a decrease in levels of maternal anxiety.\(^{8,10,18}\) Mummies will engage in tasks such as holding, transporting and eating. By developing competency and parenting roles, anxiety levels can be reduced in this way.\(^{21}\) In our study, statistical results are statistically significant in terms of the level of state concern for mothers who have not been involved in caring for their children (Table 4). In some other studies similar results were reported\(^{19-21}\)

There was no significant correlation between the anxiety levels typical of mothers and the form of children in either group, according to our findings. Erdem (2010
reported elevated rates of anxiety among mothers who had their male children at NICU. (8) In this study, the level of extreme concern among mothers with male children was believed to be a guarantee in the future due to gender and religious gender, and cultural, economic and mental understanding in Turkey. In our study, an important outcome was the unimportant relationship between maternal anxiety levels and children’s gender in terms of showing that sex did not contribute to maternal anxiety.

**Conclusion**

There was a high level of anxiety amongst mother whose newborn were admitted at NICU. There were certain causes that contributed to maternal anxiety such as lengthy stays in the NICU, the failure of the mothers to care for the infant and the lack of child health knowledge. Previous have shown that mothers need to know the nurse status of their child and to freely interact with the nurses.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**

8. Erdem Y. Anxiety levels of mothers whose infants have been cared for in unit level-I of a neonatal intensive care unit in Turkey. Journal of clinical nursing. 2010 Jun;19(11-12):1738-47.


Pathological Study of Reproductive Tracts of Awassi Ewes in Fallujah, Iraq

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Abstract

A total of forty female genital tracts of local sheep, collected from Al-Fallujah abattoir, were utilized for this study during the period from October 2018 to March 2019. Among these 20% of the samples were pregnant with out any abnormality, and 80% were non pregnant (55% without any problem and 25% showed abnormal macroscopically lesion. The main type of abnormalities were, ovarian adhesion, uterus unicornis, inactive ovaries, cystic ovaries, ovarian germ cell tumor and cervical tumor, metritis, salpingitis and hydrosalpinx with an incidence of 10%, 10%, 10%, 20%, 10%, 10%, 10%, 10% and 10% respectively. The histopathological study revealed that there were a histopathological changes in the previous cases, the ovarian giant cell tumor reported at first in this study in Iraq.

Keywords: genitalia, Awassi ewes, abnormalities

Introduction

Ewes sheep were a chief farm animals, it play an important roles in Iraq economy through provided meat, milk and wool. Unfortunately the number of sheep weakening by 42 per cent in Iraq (1). The fundamental driver of this diminishing was breakdown of the veterinary framework, including disease determination, observation and indicative administrations (2). A significant factor that affects a population’s growth rate is the fertility of its females; genital tract diseases significantly reduce flock productivity and may mean that rearing these animals is uneconomical. Several breeds of sheep were distributed all over the world. The most common breed managed and domesticated in Iraq was the Awassi breed (3,4)

Reproductive tract abnormalities of ewes resulting in infertility, subfertility or sterility cannot diagnosed by routine clinical examination so little information in ewes about the etiology of reproductive failure. Butcher house overviews of the genitalia of ewes in various parts of the world give a decent data on the sorts and frequency or pervasiveness of obsessive (inborn or obtained abnormalities (5,6).

The data on prevalence of ewes genital tract may propose the job of conceptive illnesses in constraint to sheep generation. Anyway the satisfactory learning is missing on the incidence of etiology and nature of pathological conditions in the ewe genitalia.

The present work was designed to determination the pathological change (macroscopic and microscopic) lesion of ewes genital tract abnormalities in ewes slaughtered at the slaughter house of Fallujah- Anbar province, Iraq.

Materials and Methods

This study was achieved on forty sheep female genitalia were calm one day each week in Fallujah butchery house that situated in Fallujah district, AL-Anbar province. specimens were gathered during the period from the October 2018 to March 2019. No information about the all history of the animals incorporated into this investigation. The reproductive
tract were separated after evisceration at the abattoir, the samples were transported with cool box contained normal saline to the pathology lab, college of Vet. Med. university of Fallujah, the connective tissue and the fat surrounding genitalia were evacuated beyond what many would consider possible to clear the genital organs for good examination.

Gross examination of different parts of the genitalia were done, including; ovaries, uterine horn, uterine tubes, body of the uterus, cervix, vaginal and vulva (7).

The abnormalities in different parts of the genitalia were recorded. A tissue test from ordinary and influenced some portion of genitalia (area of around one cubic centimeter) were taken and fixed in plastic compartment contained 10% formalin buffered for histopathological examination. The wax box contain biopsy were catted in 7µm in microtome, the handled and recolored done by Luna (8).

Examination of slides and the observation of changes in the abnormalities were reported.

**Results and Discussion**

Reviewed congenital and acquired pathology of the ewe reproductive system by collection extensive data from abattoir surveys. Out of forty female sheep genitalia examined, 8 (20%) were pregnant and the remain 32 (80%) were nonpregnant genitalia. The stages of pregnancy and the genitalia were appeared normal in all samples. Non pregnant ewes genitalia that give abnormal reproductive tract lesion were (10) (25%) as showed in table (1). Macroscopic lesion were includes; salpingitis, hydrosalpinx, follicular cystic ovary, ovarian fusion, luteal cystic ovary, metritis, uterus unicornis as showed in table (2) and figure (1) respectively, inactive ovaries, cervicitis also reported. These results of abnormality were higher than reported by (9,10,12,15,). Palmieri and his college in (6), they reported a similarly results; cystic structures, hypoplasia or aplasia in apart or in all genital system, our results were in disagreement with data what founded by (16). in ewes cystic ovaries isn't totally comprehended and has not gotten as much consideration as in dairy cattle, it is a typical finding in various breeds of sheep (6).

The cystic ovaries emerge because of anovulation whereby, rather than relapse, follicles continue on expanding in size and persist (17). The main cause of cystic ovaries are insufficient of LH prior or at the time ovulation (18). The incidence of inactive ovaries might be due nutrition deficiency that leads to hormonal dysfunction such as insufficient FSH, which is the primary cause of inactive ovaries (19). Ovarian tumor were observed in one case 10% from abnormalities, an histopathologic picture showed giant cell ovarian tumor figure (2E) in has been observed presence of giant cell in the ovarian stroma. It is area case and there is a little information about the occurrence of ovarian giant cell tumor. It might be due to genetic factors or other environmental cause.

Histopathological examination of fallopian tube as in Figure 2 (F,G,H) showed pus formation with in the fallopian tube mainly contained neutrophils (pyosalpingitis) arrow. high magnification showed ulcerative formation of uterine tube (ulcerative salpingitis). The obtainable study recommended that the occurrence of salpinx inflammation and hydrosalpingitis in sheep comparatively little frequency with prevalence (10% and 10%) receptivity as showed in Table (2). These discoveries are in arrangement with the result recorded bySaberivand and Haghighi (25). Adams(13) documented slight inflammation of salpinx in sheep (7%) and Ansari(20) originate 15 cases of hydrosalpingitis in 3,590 genital system of sheep which affected with our study. The low rate in ewes could be because of the valve-like move which makes impact in ewes at the utero-tubal intersection through and 3 days after estrus which assumes a role in keeping the augmentation of irresistible specialists from the uterus to the tube(22).
The histopathology exam of uterus showed that the endometrium with cystic endometrial hyperplasia (adenomyosis) figure (2I). dilation of cystic glands with preiglandular fibrosis. The available study also suggested that the incidence of metritis were (10%) which in disagreement with the results approved by (16, 26) endometritis were the greatest common outcome of uterine irregularity demonstrating that this condition theatres a more significant role in infertility. Endometritis in sheep, like in cattle, is most common in luteal phase or post-delivery and persuades embryonic harm as a result of uterine tissue disruption or through embryo cytolysis (16, 21, 27).

The histopathology exam of cervix as showed in figure (2K) (A&B), cervix with excessive formation of new glands and congestion of the blood vessels with edema and inflammatory cells in the cervical lumen and interstitial tissue. The prevalence of uterus unicornis was 10%, which might be due to genetic factors as mentioned by (28). Similar observation have been made by Vojgani(10) and Amin (12).

Finally these outcomes uncover a significant zones of reproduction cost in sheep even the nutrition are best over the time and in the nonappearance of reproductive infections and it might be credited to numerous issues like breed, organization an area specification. Such wounded are complete up of female sheep that fail to reproduct for the reason that of irregularities of the genital region (14).

It was concluded from this study that pregnant animal were slaughter in abattoir 20% while non pregnant were 80% with incidence of abnormality 25% of total sample were collected, this reflect that reproductive status of Awassi ewes in Al-Fallujah city.

### Table 1: showed the distribution of samples according to their reproduction status

<table>
<thead>
<tr>
<th>Type of genitalia</th>
<th>Total no. Sample</th>
<th>Normal genitalia</th>
<th>Abnormal genitalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy NO. %</td>
<td>8</td>
<td>8</td>
<td>0%</td>
</tr>
<tr>
<td>20%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not pregnancy No. %</td>
<td>32</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>80%</td>
<td></td>
<td>55%</td>
<td>25%</td>
</tr>
<tr>
<td>Total No. %</td>
<td>40</td>
<td>30</td>
<td>10%</td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td>75%</td>
<td>25%</td>
</tr>
</tbody>
</table>

### Table 2: showed the abnormal genitalia in non-pregnancy animal

<table>
<thead>
<tr>
<th>Abnormal genitalia</th>
<th>NO</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic ovaries</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Ovarian adhesion</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Cervical tumor</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Uterine unicornis</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Salpingitis</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Hydrosalpinx</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Metritis</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Gaint cell overian tumor</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Inactive ovaries</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>
Fig.(1): (A) photograph of Fallopian tubes showed salpingitis. (B):photograph of Fallopian tubes showed hydrosalpinx. (C):photograph of ovary showed follicular cystic ovary. (D):photograph of ovary showed ovarian fusion. (E):photograph of ovary showed luteal cystic ovary. (F):photograph of uterus showed chronic metritis. (G):photograph of uterus showed uterus unicorn.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Incidence Rate and Risk Factors of Ectopic Pregnancy at Maternity Wards in Baghdad City’s Hospitals for the Year 2019

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Abstract

Background: Ectopic pregnancy is a condition of immense gynecological importance, particularly in the developing world, because of the high morbidity and mortality associated with it and the enormous threat to life.

Objectives: To assess the incidence rate and the risk factors of ectopic pregnancy at Maternity Wards in Baghdad City’s Hospitals for the year 2019.

Methods: The study included a non-probability (convenient) sample consisted of (90) pregnant women who have ectopic pregnancies. The study subjects are recruited from 10 teaching hospitals in Baghdad, in their first trimester of gestation age. The study data is collected from pregnant women’ edical records in the Department of Statistics, data are analyzed using descriptive and inferential data analysis process.

Results: the results of the current study indicate that the incidence rate of ectopic pregnancy is (2.217) per 1000 of population at risk for the year 2019. Smoking is accounted as the most predominant risk factor for ectopic pregnancy (55.6%). Most of these pregnant women are between the ages of (15-24) years old (40.0%), college graduates (26.7%), government employees (52.2%), and have a monthly income (901.000-1.200.000) ID (32.2%).

Conclusion: Incidence rate of ectopic pregnancy at the maternity wards in Baghdad City’s hospitals is (2.217) per 1000 of population at risk for the year of 2019. The incidence rate of ectopic pregnancy has not been affected by the risk factors of infertility, previous ectopic pregnancies, IUCD, assisted reproductive techniques and smoking of ectopic pregnancy and the incidence rate of ectopic pregnancy has not been affected by pregnant demographic characteristics of age, education, occupation and monthly income.

Keywords: Incidence Rate, Ectopic pregnancy, Risk factors.

Introduction

An ectopic pregnancy occurs when a fertilized egg grows outside the uterus [1]. Ectopic pregnancy is a life threatening emergency, and if not treated, it can produce adverse effect. It remains the leading cause of pregnancy related death during the first trimester, where it is responsible for 9 – 10 % of all maternal deaths [2]. The incidence of EP has doubled or trebled over the last 20 years and has become a major public health problem. EP accounts for approximately 2% of reported pregnancy. Approximately 1/100 pregnancies are ectopic, with the concepts usually implanting in the fallopian tube. Some ectopic pregnancies resolve spontaneously, but others continue to grow and lead to rupture of the tube [3]. Important etiological factors for EP are pelvic inflammatory disease (PID), post-aborted sepsis, puerperal sepsis, previous EP, previous pelvic surgery and the uses of contraceptive device.

Presence of history of infertility and congenital defects of fallopian tubes consider also as other risk
The present study aims to estimate the incidence of ectopic pregnancy among Iraqi women in Baghdad City as it is one of the Ministry of Health research plans priorities, in order to provide data that can assist in the health strategies planning of control.

**Methodology**

A descriptive analytic element design was conducted throughout the present study among pregnant women at Maternity department attend hospitals in Baghdad City. Department of Statistics (Medical records), in ten hospitals at Baghdad city. Determined validity through panel of experts and reliability of questionnaire(Alpha Correlation Coefficient) is (0.83) which considered significant through of pilot study, they are measured as (2) for Yes and (1) for no.

**Results and Discussion**

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 - 24</td>
<td>36</td>
<td>40.0</td>
</tr>
<tr>
<td>25 - 34</td>
<td>34</td>
<td>37.8</td>
</tr>
<tr>
<td>35 - 44</td>
<td>17</td>
<td>18.9</td>
</tr>
<tr>
<td>45 - 54</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>100.0</td>
</tr>
</tbody>
</table>

| 2. Education                |           |         |
| Illiterate                  | 2         | 2.2     |
| Able to Read and Write      | 2         | 2.2     |
| Primary school Graduate     | 12        | 13.3    |
| Intermediate School Graduate| 17        | 18.9    |
| Secondary School Graduate   | 12        | 13.3    |
| Institute (Diploma) Graduate| 17        | 18.9    |
| College Graduate            | 24        | 26.7    |
| Post Graduate               | 4         | 4.4     |
| Total                       | 90        | 100.0   |

| 3. Occupation               |           |         |
| Government Employee         | 47        | 52.2    |
| Self-Employed               | 17        | 18.9    |
| Unemployed                  | 26        | 28.9    |
| Total                       | 90        | 100.0   |

| 4. Monthly Income           |           |         |
| 300.000 - 600.000 ID        | 23        | 25.6    |
| 601.000 - 900.000 ID        | 17        | 18.9    |
| 901.000 - 1.200.000 ID      | 29        | 32.2    |
| 1.201.001- 1.500.000 ID     | 21        | 23.3    |
| Total                       | 90        | 100.0   |

Results, out of this table, depicts that most of these pregnant are (15-24) year old (40.0%), college graduates (26.7%), government employee (52.2%) and making monthly income of (901.000-1.200.000) ID (32.2%).
\[
\text{Incidence Rate} = \frac{\text{New Cases}}{\text{Total Population}} \times 1000
\]

\[
= \frac{90}{40500} \times 1000
\]

\[
= 2.217
\]

Figure (1): Incidence Rate of Ectopic Pregnancy in Baghdad City during 2019.

Table (2): Risk Factors for Ectopic Pregnancy

<table>
<thead>
<tr>
<th>Lists</th>
<th>Risk Factors</th>
<th>Yes</th>
<th></th>
<th>No</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frequency</td>
<td></td>
<td>Percent</td>
</tr>
<tr>
<td>1</td>
<td>Infertility</td>
<td>39</td>
<td>43.3</td>
<td>51</td>
<td>56.7</td>
</tr>
<tr>
<td>2</td>
<td>Previous ectopic pregnancies</td>
<td>12</td>
<td>13.3</td>
<td>78</td>
<td>86.7</td>
</tr>
<tr>
<td>3</td>
<td>IUCD</td>
<td>24</td>
<td>26.7</td>
<td>66</td>
<td>73.3</td>
</tr>
<tr>
<td>4</td>
<td>Assisted Reproductive Techniques</td>
<td>24</td>
<td>26.7</td>
<td>66</td>
<td>73.3</td>
</tr>
<tr>
<td>5</td>
<td>Smoking</td>
<td>50</td>
<td>55.6</td>
<td>40</td>
<td>44.4</td>
</tr>
</tbody>
</table>

Results, out of this table, show that smoking is accounted as the most risk factor for ectopic pregnancy (55.6%) in these women.

Table (3): The Relationship between Incidence Rate of Ectopic Pregnancy and Risk Factors of Ectopic Pregnancy

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t-test</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Standard Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>22.178</td>
<td>0.000</td>
<td>124976.342</td>
<td>0.000</td>
</tr>
<tr>
<td>Risk Factors</td>
<td>-2.083E-5</td>
<td>0.000</td>
<td>-0.084</td>
<td>-0.789</td>
</tr>
</tbody>
</table>

a. Dependent Variable: Incidence Rate

Result, out of this table, indicates that there is no significant relationship between incidence rate of ectopic pregnancy and risk factors of ectopic pregnancy.
Discussion of the Study Results

Part I: Discussion of Pregnant Socio-demographic Characteristics

Analysis of such characteristics has depicted that these pregnant women are mostly teenagers (15-24) year old and they are accounted for (40.0%) of the total sample (Table 4-1). This finding presents an evidence that such pregnant in this age can develops ectopic pregnancy than others and they are presenting the nature of pregnant women in our culture of getting marriage at earlier age.

The current study finding agrees with a hospital based unmatched case control study results, of (99) cases with ectopic pregnancy and (200) controls who have been selected with a simple random sampling method, the study has identified that the average (± SD) of pregnant age is 27 (± 5) years [4].

Information about maternal demographic characteristics is obtained from the Pregnancy Risk Assessment Monitoring System by (CDC, 2009) has agreed with the study finding and presented evidence that the age distribution of respondents is as follows: (10.5%) are (19) years of age and younger, (76.2%) are (20–34) years of age, and (13.3%) are (35) years old and older.

With respect to their education, the study findings indicate that most of these pregnant are well educated as being college graduates (26.7%) (Table1). This can be interpreted in a way that women in our society are fortunate to continue and pursue well education in the recent years.

Such result does not agree with a report has indicated that more than half (53.4%) of pregnant women reported having more than a high school education, followed by (27.9%) with at least a high school education and (18.7%) with less than a high school education [5].

The current study finding is agreed with that of a cross-sectional study, of (300) pregnant woman with ectopic pregnancy who are with only primary/Junior High School education (p=0.048) [6].

With regard to their employment, the study has revealed that more than half of the study sample are government employee (52.2%) and making monthly income of (901.000-1.200.000) Iraqi Dinar (32.2%) (Table1). Such findings can be inferred that these pregnant play the role as breadwinners for their families.

In the contrary with a retrospective study in Urban Nigeria, on (72) case of ectopic pregnancy, shows that the health problem of ectopic pregnancy is exacerbated with social issues leading to financial stress due to palpable poverty[7].

Part II: Discussion of the Incidence Rate of Ectopic Pregnancy

The present study reveals that the incidence rate of ectopic pregnancy is 2.217 per 1000 of population at risk for the year of 2019 in Baghdad City (Figure 1). It is worth to mention that there are other cases of ectopic pregnancy (about 50 cases) which was documented, but the researcher couldn’t reach their files due to the uncooperative administration of the hospitals under the study.

This finding is higher than a finding of retrospective study, six thousand six hundred sixty-two women give birth in the hospital, (88) women are diagnosed with ectopic pregnancy with an incidence of 1.3% [8].

It has been found in the literature that ectopic pregnancy is a potentially life-threatening condition occurring in (1-2 %) of all pregnancies. The most common ectopic implantation site is the fallopian tube, though 10 % of ectopic pregnancies implant in the cervix, ovary, and myometrium, interstitial portion of the fallopian tube, abdominal cavity or within a cesarean section scar [9].

It has been noted that the incidence rate is (0.5%-1.5%) of all pregnancies. Even though its incidence rate is drop off when compared with earlier decades, it is still the foremost causes of maternal morbidity and mortality in the first trimester of pregnancy, especially in developing countries. In Pakistan, it varies from 1:1 124 to 1:130 pregnancies [10].

Furthermore, the current study is more far lower than a worldwide view presents that incidence rate is (12.4/1000) reported pregnancy in England-Wales, (0.52/1000) women of reproductive age in Beijing-China, (1.68/100) total births in Nigeria and (4%) in
Part III: Discussion of Risk Factors for Ectopic Pregnancy

Analysis of risk factors results for ectopic pregnancy reveals that smoking is accounted as the most risk factor for ectopic pregnancy (55.6%) and then comes the risk factor of infertility (43.3%), and the least risk factors are IUCD (26.7%) and assisted reproductive techniques (26.7%) and previous ectopic pregnancies (13.3%) (Table 4-7). The findings of the study have presented evidence about these factors as being considered as risk factors for ectopic pregnancy among the study sample.

Such findings agrees with a matched case-control study, of (88) cases and (176) controls, has explored that of the fifteen identified risk factors, four are independently associated with increased odds of ectopic pregnancy: prior pelvic inflammatory disease (PID) (adjusted odds ratio [AOR] 13.18; 95% CI 6.19–27.42), followed by current use of levonorgestrel-only pills for emergency contraception (LNG-EC) (AOR 10.15; 95% CI 2.21–46.56), previous use of depot medroxy progesterone acetate (DMPA) (AOR 3.01; 95% CI 1.04–8.69) and smoking at the time of conception (AOR 2.68; 95% CI 1.12–6.40) [12].

A case-control study, of women who are diagnosed with ectopic pregnancy (n = 2411) and women with intrauterine pregnancies (n = 2416) are recruited from five hospitals in Shanghai, China reveals that the risk of ectopic pregnancy is associated with the traditional risk factors including previous EP (Adjusted odds ratio [AOR] = 2.72, 95% CI: 1.83–4.05), previous Chlamydia trachomatis infection (Adjusted OR = 3.18, 95% CI: 1.66–2.88), previous infertility (AOR = 2.18, 95% CI: 1.66–2.88), previous adnexal surgery (AOR = 2.09, 95% CI: 1.49–2.93), previous appendectomy (AOR = 1.64, 95% CI: 1.13–2.37), and previous use of intrauterine devices (IUDs) (AOR = 1.72, 95% CI: 1.39–2.13). Additionally, EP risk is increased following the failure of most contraceptives used in the current cycle including IUDs (AOR = 16.43, 95% CI: 10.42–25.89), oral contraceptive pills (AOR = 3.02, 95% CI: 1.16–7.86), levonorgestrel emergency contraception (AOR = 4.75, 95% CI: 3.79–5.96), and female sterilization (AOR = 4.73, 95% CI: 1.04–21.52). Stratified analysis shows that in vitro fertilization and embryo transfer (IVF-ET) is the main risk factor for EP in women with tubal infertility (AOR = 8.99, 95% CI: 1.98–40.84), although IVF-ET shows no association with EP in women with non-tubal infertility (AOR = 2.52, 95% CI: 0.14–44.67) [13].

In a mini-review study, it has been reported that risk factors associated to ectopic pregnancy are pelvic inflammatory disease, past history of miscarriages, age, parity, infertility, previous ectopic pregnancy, induction of ovulation and intrauterine device usage [10].

A cross-sectional study, of (900) pregnant woman diagnosed with ectopic pregnancy, has depicted that the risk of EP is associated with previous adnexal surgery (adjusted OR = 3.99, 95% CI: 2.40–6.63), uncertainty of previous pelvic inflammatory disease (adjusted OR = 6.89, 95% CI: 3.29–14.41), and positive CT IgG serology (adjusted OR = 5.26, 95% CI: 3.94–7.04). A history of infertility including tubal infertility (adjusted OR = 3.62, 95% CI: 1.52–8.63), non-tubal infertility (adjusted OR = 3.34, 95% CI: 1.60–6.93), and in vitro fertilization (IVF) treatment (adjusted OR = 5.96, 95% CI: 1.68–21.21) are correlated with the risk of ectopic pregnancy [14].

A case-control study, of (150) cases and (300) controls, has found that the risk of ectopic pregnancy has increased with the use of intrauterine device and tubal ligation, whereas decreased with use of oral contraception [15].

A five year retrospective study, of (72) case of ectopic pregnancy, reveals that related risk factors include pelvic inflammatory disease, previous history of abortions, infertility and a previous history of ectopic pregnancy [7].

A hospital based unmatched case control study, of (99) cases with ectopic pregnancy and (200) controls have been selected with simple random sampling method, has indicated that the risk factors identified are marital status and history of contraception use. Accordingly, women with single marital status are (10.81) times (95% CI AOR (3.601, 32.465) more likely to develop ectopic pregnancy than married once. Those who use contraception are (2.27) times (95% CI, AOR (0.214, 24.02) more likely to develop ectopic pregnancy than who do not use contraception (Kebede and Dessiein 2018) [4]. This study is not compatible with current study.
Part V: Discussion of the Relationship between Incidence Rate of Ectopic Pregnancy and Risk Factors of Ectopic Pregnancy

The study findings indicate that the incidence rate of ectopic pregnancy has not been affected by the risk factors of infertility, previous ectopic pregnancies, IUCD, assisted reproductive techniques and smoking of ectopic pregnancy (Table 4-10).

Contrary findings have been reported in the literature that risk factors of surgical, gynecological, obstetrics, sexual, contraceptive, and infectious histories; demographic characteristics; smoking habits; and fertility markers are initiated to be associated with the incidence of ectopic pregnancy.[16]

Conclusion

Incidence rate of ectopic pregnancy at the maternity wards in Baghdad City’s hospitals is (2.217) per 1000 of population at risk for the year of 2019, The incidence rate of ectopic pregnancy has not been affected by the risk factors of infertility, previous ectopic pregnancies, IUCD, assisted reproductive techniques and smoking of ectopic pregnancy and the incidence rate of ectopic pregnancy has not been affected by pregnant demographic characteristics of age, education, occupation and monthly income.

Recommendations:

Measures for early diagnosis of ectopic pregnancy, Annual measurements for the incidence of ectopic pregnancy and accurate follow up for registration and documentation of the cases.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Lip Thickness with Tongue Space Area Assessment By Cephalometric Analysis for Iraqi Adult Sample with Class II Dental and Skeletal Pattern

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Abstract

Background: With different skeletal classes, there may be some variability among the lip thickness and tongue space area that could be the causative factor for skeletal malrelationship so that this study was aimed to determine the tongue and the available boundary space area with lip thickness and correlate them together in sample with class II skeletal pattern.

Methods: Seventy Iraqi adult subjects (32 males and 38 females) with cl II skeletal pattern who have increased over jet and ANB angle greater than four degrees. The cephalometric measurements include upper and lower lip thicknesses with height and length of tongue and position of tongue base and hyoid bone from the cervical line. The cephalograms were analyzed using AutoCAD program to measure demanded areas.

Results: This study revealed that a significant gender difference was found between T-Area, V-TT, TH, V-FP, AH-CL, AH-FP, and upper and lower lip thickness in which higher mean value in males than females. The correlation between all the measured variables of present sample with SNA, SNB and ANB angles a non-significant difference was found except for V-CL which shows significant difference. Strong correlation was found among the T-Area with V-TT, TH, V-FP, AH-CL, AHFP, upper lip thickness and lower lip thickness.

Conclusions: In skeletal class II, the associated cranial base angles shows independent association from the boundary soft tissues so that these variations not always contribute to be the causative factor for skeletal disharmony.

Keywords: class II, Cephalometric, lip thickness, tongue space.

Introduction

Evaluation of soft tissue considered the integral part in diagnosis and treatment planning. As the tongue size influence the development of dentoalveolar structures (1), in class II skeletal relationships the small size of the tongue may influence the size of mandible or the positioning of dentoalveolar structures.

The skeletal mal-relationship in craniofacial growth and development may be caused and controlled by both genomic and epigenetic effects. According to Moss theory of growth, the soft tissues grow, and both bone and cartilage react (2,3). The airway form intraorally is influenced by the tongue form and size (4). The major part of upper airway is formed by the tongue which has both extrinsic and intrinsic muscles (5)(6).

Some previous studies have assessed the measurements of the tongue space area in skeletal class I and others measure the tongue size and airway space in patients with obstructive sleep apnea in various malocclusions (7,8,9). Other studies have assessed measurements of soft tissue norm as lip thickness in skeletal class I pattern (10,11,12). The aim of this study was to determine the tongue and the available boundary...
space area with lip thickness in patients with skeletal class II and correlate them together as by understanding these correlations among hard and soft tissues we can predict the final outcome and stability of orthodontic treatment.

**Materials and Methods**

The sample of this retrospective study was carried out at department of orthodontics/ college of dentistry/ Baghdad University, and included seventy Iraqi adult participants (32 males and 38 females) who seeking orthodontic treatment with an age range between 18-28 years to exclude the effect of growth. A true lateral cephalogram were taken for each subject in this sample.

The sample were selected according to the following criteria:

14. Full permanent teeth except 3rd molars.
15. Class II skeletal relationship with increased over jet and ANB angle greater than four degrees.
18. Absence of acquired or congenital abnormalities to the face.

Each radiograph was taken with Frankfort horizontal parallel to the floor with the teeth in centric occlusion and the eyes straight look ahead.

The x-ray Machine used for this study was DIMAX3 Planmeca radiographic machine (digital x-ray unit system) and the digital cephalograms obtained were analyzed with AutoCAD 2015 in which the magnification of the images obtained was corrected by measuring the magnification factor of the cephalometric machine involving the nasal rod ruler graduation and then the measurements were gained.

The measurements of hard and soft tissues include evaluation of the tongue and boundary space region with lip thicknesses according to the landmarks, planes, linear and area measurements used in this study summarized in table 1 and described in figures 1,2 and 3.

<table>
<thead>
<tr>
<th>Planes, lines and areas</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frankfort plane (FP)</td>
<td>This plane extended from orbitale (Or) to porion (Po).</td>
</tr>
<tr>
<td>Cervical line (CL)</td>
<td>The line extension on second and third cervical vertebrae anteriorly.</td>
</tr>
<tr>
<td>V-TT</td>
<td>The line from the tongue tip to the tongue base (it represent the tongue length).</td>
</tr>
<tr>
<td>TH</td>
<td>Distance from ST point perpendicular to VTT. (it represent the tongue height).</td>
</tr>
<tr>
<td>V-FP</td>
<td>The line from V point perpendicular to FP.</td>
</tr>
<tr>
<td>V-CL</td>
<td>The line form V point to CL parallel to the FP.</td>
</tr>
<tr>
<td>AH-FP</td>
<td>The line from anterior tip of hyoid perpendicular to FP.</td>
</tr>
<tr>
<td>AH-CL</td>
<td>Line from anterior tip of hyoid to the cervical line parallel to FP.</td>
</tr>
<tr>
<td>Upper lip tissue thickness</td>
<td>The line measured between middle point at facial surface of upper central incisors to upper lip anterior.</td>
</tr>
<tr>
<td>Lower lip tissue thickness</td>
<td>The line measured between middle point at facial surface of lower central incisors to lower lip anterior.</td>
</tr>
<tr>
<td>Tongue area</td>
<td>This area formed by line passing through tip of the tongue, ST point and V point which include the tongue boundaries.</td>
</tr>
</tbody>
</table>
Figures 1: Cephalometric landmarks involved in this study.

Figures 2: Cephalometric radiograph reveals the planes and tongue area used in this study.

Figures 3: Cephalometric radiograph showing linear measurements used in this study.
Statistical Analysis

By the using of the Statistical Package for Social Sciences (SPSS) version 20, the 76 statistical analysis was done in which all data of sample were calculated.

The statistical analysis included:

1- Descriptive Statistics: including mean, standard deviation, minimum and maximum values.

2- Inferential Statistics: including

- Independent sample t-test: for the genders difference comparison.
- Sig. (2-tailed) or P value.
- Pearson’s Correlation Coefficient:

In order to quantify the strength of the association between angles, linear and area measurements; Pearson’s correlation coefficients were used.

For the statistical evaluation of the results, the following levels of significance are used: p≥0.05 Non-significant (NS)
0.05>p≥0.01 * Significant (S)

Results

The descriptive statistics with gender difference of the present sample was described in table 2, in which a non-significant difference was found among SNA, SNB, ANB and V-CL. However, for the other variables the T-Area, V-TT, TH, V-FP, AH-CL, AH-FP, upper and lower lip thickness a significant difference was found between both genders in which higher mean was shown in males in all measured variables except for ANB angle.

Table 2: Gender difference of the present sample (Male N= 32 and Female N= 38).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>T-test</th>
<th>Sig. (2tailed)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNA</td>
<td>M</td>
<td>32</td>
<td>83.281</td>
<td>78.0</td>
<td>91.0</td>
<td>2.9319</td>
<td>.760</td>
<td>.450</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>38</td>
<td>82.71</td>
<td>71.0</td>
<td>87.0</td>
<td>3.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNB</td>
<td>M</td>
<td>32</td>
<td>76.03</td>
<td>72.0</td>
<td>84.0</td>
<td>2.56</td>
<td>1.003</td>
<td>.319</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>38</td>
<td>75.37</td>
<td>67.0</td>
<td>80.0</td>
<td>2.91</td>
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<tr>
<td>ANB</td>
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<td>32</td>
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<td>10.0</td>
<td>1.81</td>
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<td>.851</td>
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<tr>
<td></td>
<td>F</td>
<td>38</td>
<td>7.34</td>
<td>1.0</td>
<td>11.0</td>
<td>2.21</td>
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<tr>
<td>T-Area (mm^2)</td>
<td>M</td>
<td>32</td>
<td>2710.594</td>
<td>260.2531</td>
<td>2182.0</td>
<td>3145.0</td>
<td>6.193</td>
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<td>2043.0</td>
<td>2726.0</td>
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</table>

Legend:
- Yellow: V-TT
- Red: TH
- Dark green: V-FP
- Light green: V-CL
- Dark blue: AH-FP
- Light blue: AH-CL
The correlation between tongue area, upper and lower lip thickness and related linear measurements with the others was described in table 3 as the following:

- strong correlation was found among the **T-Area**, V-TT, TH, V-FP, AH-CL, AH107 FP, upper lip thickness and lower lip thickness.

- strong correlation was found among the **ANB angle with SNA, SNB and V-CL.** 109, strong correlation was found among the **V-CL with ANB, AH-CL, upper lip 110 thickness and lower lip thickness.**
Table 3: Correlation between tongue area, upper and lower lip thickness and related linear measurements with the others. (N=70)

<table>
<thead>
<tr>
<th></th>
<th>TAREA (mm².)</th>
<th>SNA</th>
<th>SNA</th>
<th>ANB</th>
<th>V-TT (mm.)</th>
<th>TH (mm.)</th>
<th>V-FP (mm.)</th>
<th>V-CL (mm.)</th>
<th>AHCL</th>
<th>AH-FP (mm.)</th>
<th>upper lip</th>
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<tr>
<td>SNA</td>
<td>r</td>
<td>.146</td>
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<td>.286</td>
<td>.049**</td>
<td>.495**</td>
<td>.174</td>
<td>.000</td>
<td>.000</td>
<td>.690</td>
<td>.149</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>.259</td>
<td>.229</td>
<td>.286</td>
<td>.049**</td>
<td>.495**</td>
<td>.174</td>
<td>.000</td>
<td>.000</td>
<td>.690</td>
<td>.149</td>
</tr>
<tr>
<td>SNB</td>
<td>r</td>
<td>.129</td>
<td>.769**</td>
<td>.286</td>
<td>.049**</td>
<td>.495**</td>
<td></td>
<td>.000</td>
<td>.000</td>
<td>.690</td>
<td>.149</td>
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<tr>
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<td>.174</td>
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<tr>
<td>V-TT (mm.)</td>
<td>r</td>
<td>.568**</td>
<td>.128</td>
<td>.004</td>
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<td>.229</td>
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<td>.049**</td>
<td>.495**</td>
<td></td>
<td>.000</td>
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<td>.690</td>
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<tr>
<td>TH (mm.)</td>
<td>r</td>
<td>.735**</td>
<td>.057</td>
<td>.121</td>
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<td>.248**</td>
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<td>p</td>
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<td>.229</td>
<td>.286</td>
<td>.049**</td>
<td>.495**</td>
<td></td>
<td>.000</td>
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<tr>
<td>V-FP (mm.)</td>
<td>r</td>
<td>.670**</td>
<td>-.013</td>
<td>.102</td>
<td>-.159</td>
<td>.466**</td>
<td>.763**</td>
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<td>.286</td>
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<td>.495**</td>
<td></td>
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</tr>
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<td>V-CL (mm.)</td>
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<td>-.069</td>
<td>.105</td>
<td>-.249**</td>
<td>-.056</td>
<td>.139</td>
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<td>.000</td>
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<td>AHCL</td>
<td>r</td>
<td>.107</td>
<td>.571</td>
<td>.388</td>
<td>.038</td>
<td>.648</td>
<td>.251</td>
<td>.204</td>
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<td>.149</td>
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<td>AH-FP (mm.)</td>
<td>r</td>
<td>.534**</td>
<td>-.116</td>
<td>.001</td>
<td>-.179</td>
<td>.369**</td>
<td>.393**</td>
<td>.489**</td>
<td>.594**</td>
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<tr>
<td>p</td>
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<td>.286</td>
<td>.049**</td>
<td>.495**</td>
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<td>.000</td>
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<td>.149</td>
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<td>upper lip</td>
<td>r</td>
<td>.354**</td>
<td>.044</td>
<td>.085</td>
<td>-.048</td>
<td>.158</td>
<td>.463**</td>
<td>.518**</td>
<td>.353**</td>
<td>.376**</td>
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<td>.000</td>
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<td>.690</td>
<td>.149</td>
</tr>
<tr>
<td>lower lip</td>
<td>r</td>
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<td>.018</td>
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<td>.345**</td>
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<td></td>
<td>.000</td>
<td>.000</td>
<td>.690</td>
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</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed). 117  
*. Correlation is significant at the 0.05 level (2-tailed)

Discussion

The Harmony of soft tissue profile in some conditions difficult to obtain which may be 121 due to soft tissue covering the teeth and bones is highly variable in their thickness. The 122 thickness and tension of facial soft tissues in addition to the imbalance of dental and 123 skeletal structures could lead to these variations among soft tissue profile (14). Some 124 authors have suggested that the using of cephalograms is easier than the other methods 125 for airway patency measurement (15). Parkkinen et al also confirm that the lateral 126 cephalogram is a reliable method for measuring the dimensions of the nasopharyngeal 127 and retropalatal areas (16,17).

128 In this study, the tongue space area for males and related linear measurements in class 129 II pattern showed higher mean values than females for most measured variables. A 130 significant gender difference was found among the T-Area, V-TT, TH, V-FP, AH-CL, 131 AH-FP,
which is in coincidence with class 1 sample (for the same ethnic population) 132 by other study (7).

133 For the lip thickness, a significant gender difference was found in which higher mean 134 values was in males than females for both upper and lower lips which agrees with class

1 sample (for the same ethnic population) by Al-Janabi MF and Kadhom Z (11) as described in table 4. The upper lip is thinner than the lower lip which differs from class 1 sample by Al-Janabi MF and Kadhom Z who found thicker upper lips especially for females with the same age group. This may be related to the angulation of incisors in class 2 subjects as it is considered the most important determinants to the related soft tissues (6,18). Tatjana Perović ans Zorica Blažej use Burstone lines to compare facial soft tissue thickness among class 2 skeletal pattern (19); they presented that in class 2 skeletal relation the soft tissue thickness at the upper lip is thinner than the lower for both genders which agrees with class 2 sample of this study. However, the gender difference reveals a non-significant difference for class 2 skeletal pattern which disagrees with this study as it could be related to ethnic variation. Kristina Lopatienė et al reveal that the upper lip thickness in skeletal class 2 the females have higher mean value than males 148 (20). The gender difference; however, reveal a non-significant difference among the class 149 2 subjects (tab. 5).

**Table 4: comparative data of class II sample of present study with normative data (class I) of the same ethnic population by other studies.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>present study (Cl II)</th>
<th>Kadhum MA (Cl I)</th>
<th>Al-Janabi MF and Kadhom Z (Cl I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-AREA (mm²)</td>
<td>M</td>
<td>2710.594</td>
<td>2551.67</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2369.605</td>
<td>2168.60</td>
<td>-</td>
</tr>
<tr>
<td>V-TT (mm)</td>
<td>M</td>
<td>75.27</td>
<td>76.08</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>72.52</td>
<td>68.38</td>
<td>-</td>
</tr>
<tr>
<td>TH</td>
<td>M</td>
<td>38.67</td>
<td>33.46</td>
<td>-</td>
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<td></td>
<td>F</td>
<td>32.64</td>
<td>28.93</td>
<td>-</td>
</tr>
<tr>
<td>V-FP</td>
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<td></td>
<td>F</td>
<td>76.94</td>
<td>69.55</td>
<td>-</td>
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<tr>
<td>V-CL</td>
<td>M</td>
<td>20.36</td>
<td>19.98</td>
<td>-</td>
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<tr>
<td></td>
<td>F</td>
<td>18.84</td>
<td>16.45</td>
<td>-</td>
</tr>
<tr>
<td>AH-CL</td>
<td>M</td>
<td>33.84</td>
<td>34.14</td>
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<td>30.37</td>
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<td>-</td>
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<tr>
<td>AH-FP</td>
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<td>90.39</td>
<td>87.32</td>
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<td></td>
<td>F</td>
<td>78.97</td>
<td>72.53</td>
<td>-</td>
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<td>Upper lip</td>
<td>M</td>
<td>12.48</td>
<td>-</td>
<td>13.57</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10.95</td>
<td>-</td>
<td>12.29</td>
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<tr>
<td>Lower lip</td>
<td>M</td>
<td>13.60</td>
<td>-</td>
<td>14.26</td>
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<tr>
<td></td>
<td>F</td>
<td>12.18</td>
<td>-</td>
<td>12.18</td>
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</table>
of the association between tongue area, upper and lower lip thickness and related linear measurements with the others was described in table 3 as the following:

- The strong correlation of the T-Area, V-TT, TH, V-FP, AH-CL, AH-FP with each other, this may be related to their representation to the tongue extensions as it lies within the same region.

By meaning of soft tissue area of the tongue influences the boundary hard tissues.

- The strong correlation among the AH-CL with T-Area, TH, V-TT, V-FP, V-CL and AH-FP could be explained by their regional relation with the tongue base in this region.

- The strong correlation among the V-CL with ANB, AH-CL, upper and lower lip thickness may be related to their association to the hard tissues skeletal bases.

- The strong correlation among the upper and lower lip thickness with each other as the lips usually follows the teeth and skeletal base at the associated region which is in agreement with Manal AShamlan and Abdullah M Aldrees (21) who suggest that is due to variation in the upper incisors location with the alveolar bone on the dental base region and the sagittal inclination and position of the lower incisors.

- The strong correlation among the upper and lower lip thickness with T-Area, TH, VFP, V-CL, AH-CL and AH-FP suggest this relation due to the compensatory balance of hard tissue in skeletal class 2 between the tongue and associated inner structures from one side and the upper and lower lips from other side.

However, the correlation among SNA, SNB and ANB angles were strong among each other but weak correlation with the other variables. This suggests that these associated cranial base angles may have independent association from the boundary soft tissue area like the tongue. This could be due to variability of muscular activity and soft tissue tension to the nearby dentoskeletal structures.

**Conclusion**

The tongue space area with lip thickness and related linear measurements in skeletal class II pattern for males showed higher mean values than females for most measured variables and the associated cranial base angles shows independent association from the boundary soft tissue of tongue area and this may be related to variations of thickness and tonicity of facial muscles so that these variations may not always contribute to be the causative factor for skeletal disharmony.

**Ethical Clearance:** The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding.

**References**

9. Al-Khawaja NF, Kadhom ZM, Al-Tuma RR. Soft tissue cephalometric norms for a sample of Iraqi


Synthesis, Characterization and Anti-Inflammatory Study of New Heterocyclic Coumarin Derivatives

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Abstract

The inflammation is one of the most central processes in animal cells defense versus certain injuries or infections of microbes. The most essential metabolic precursor for many inflammatory pathways is PGH2 (prostaglandin), which catalytic synthesis from the AA (arachidonic acid) by COX enzymes. COX can be divided into three isomer COX-1 has important role in many physiological function like hemostasis, platelet aggregation, and protection of gastric mucosa, COX-2 when stimulate cause formation of PGE2 excessively, with other prostaglandin then decrease the pain threshold and nerve ending sensitization, which induce pain, increase permeability of vascular and then enhance the inflammatory associated diseases pathway, and COX-3 has special characteristic, its higher sensitivity to acetaminophen and present in brain. The NSAIDs are therapeutic agents used for the treatment of inflammation, pain, and fever, they work by decreasing the production of prostaglandins due to inhibiting the function of the cyclooxygenase (COX) enzyme, we have two types nonselective and selective COX-2 inhibitors. In order to design new agents with no or low side effect, the COX-2 selectivity should be increased, this achieved by design molecule structurally similar to approved selective COX-2 inhibitors. The synthetic compound in this study contain three pharmacophores, a nucleus of coumarin and substituted oxazole moiety, separated by a hydrazonoethyl spacer, which have structural similarity properties to selective COX-2 inhibitors.

Keywords: Heterocyclic coumarin derivatives, anti-inflammatory, selective COX-2 inhibitor

Introduction

Inflammation is one of the most central processes desired in defense of animal cells versus certain injuries or infections of microbes. Though, inflammation orderly progresses to acute and chronic. Chronic inflammation is caused by a set of diseases like cardiovascular diseases, cancer, and neurodegenerative disorders (¹). The driving force for accumulation of fluid may rise only from the actual tissue metabolic activity, the final being a balance between anabolic and catabolic processes in physiological conditions (²). Main proceedings occur through the inflammatory process contain alteration of vascular permeability, recruitment of leukocyte and accumulation, and liberation inflammatory mediator release (³). Inflammation is a popular pathogenesis of numerous chronic diseases, such as cardiovascular diseases, arthritis, diabetes, bowel disease and cancer (⁴). The most essential metabolic precursor for many inflammatory pathways is the AA (arachidonic acid). many internal and external factor can activate the PLA2 (phospholipase A2). This activation split bounded AA from the membrane phospholipids and makes it ready for the major three inflammatory pathways, which are lipoxygenase, COX pathway, and cytochrome P-450 monooxygenase (⁵). COX can be divided into, COX-1 has important role in many physiological function like hemostasis, platelet aggregation, and protection of gastric mucosa (⁶). The stimulation of COX-2 cause formation of PGE2 (major metabolic product) excessively, with other prostaglandin then decrease the pain threshold and nerve ending sensitization, which induce pain, increase permeability of vascular and then enhance the inflammatory associated diseases pathway (⁷). COX-
3 has special characteristic, its higher sensitivity to acetaminophen, and it has been reported in brain and heart (7). NSAIDs are remarkable therapeutic agents used for the treatment of inflammation, pain, and fever. They work by decreasing the production of prostaglandins due to inhibiting the function of the cyclooxygenase (COX) enzyme (8). The traditional types are correlated with side effects like ulceration of gastrointestinal (GI) and renal toxicity because of their inhibition of COX-1 pathway (nonselective) (9). coumarins consisting of a benzene ring connect to a pyrone ring, figure (1), so they are classified as a type of the benzopyrone family (10,11).

**Figure (1) Chemical structure of Coumarin**

Both natural and synthetic origin compounds of coumarins have an important. Several compounds which containing the coumarin nucleus show useful and varied biological and pharmaceutical activities, Some of these compounds useful in antitumor, photochemotherapy, anti-HIV therapy, antibacterial, antifungal, anticoagulants, antioxidant agents, as CNS-stimulants, and as dyes. The synthetic, semi-synthetic and natural coumarins are beneficial substances in drug designs and studies (12,13). This work is focus on Synthesis and anti-inflammatory evaluation of coumarins derivatives containing three pharmacophores a nucleus of coumarin and substituted oxazole moiety, separated by a hydrazonoethyl spacer.

**Materials and methods**

3-acetyl coumarin bought from SRCT company (Shanghai). Solvent and other reagent that used through reaction were bought from the chemicals store of college of the pharmacy. The monitoring of the reactions was done by thin layer chromatography (TLC), the mobile phase solvent systems used are A: toluene:ethyl (2:1) and B: chloroform:methanol (9.5:0.5). Electronic melting point apparatus (Stuart SMP30) was used to determine all melting points in this study. FTIR spectrophotometer (Schemadzu, Japan), were done by thin film technique. 1HNMR spectra were obtained on BRUKER model Ultra shield 500 MHz spectrophotometer, using Dimethyl sulfoxide (DMSO) as a solvent.

The pathway of synthesis for final compounds was illustrated below in scheme (1)

**Scheme (1) the overall pathway of synthesis for final compounds**
Synthesis of (E)-2-(1-(2-oxo-2H-chromen-3-yl) ethylidene) hydrazine-1-carboxamide compound (I)

A mixture of 3-acetyl coumarin (1.88 g, 0.01 mol), hydrazine-carboxamide.HCl (1.11 g, 0.01 mol), and anhydrous sodium acetate (0.82 g, 0.01 mol) in (25 mL) absolute ethanol was refluxed for 3–4 hrs. The obtained solid was filtered, dried, and recrystallized from ethanol to give compounds (I) (14, 15, 16).

Yield = 89%, Rf = 0.42(B), IR: (3483 & 3147 cm\(^{-1}\)) N-H stretching of 1º & 2º amine, (3074 cm\(^{-1}\)) C-H stretching of Ar ring, (2974 cm\(^{-1}\)) C-H stretching of alkene, (1747 cm\(^{-1}\)) C=O stretching of lactone ring, (1705 cm\(^{-1}\)) C=O stretching of amid, (1585 cm\(^{-1}\)) C=N stretching, (1608, 1492 & 1435 cm\(^{-1}\)) C=C stretching of Ar ring (1238 & 1265 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1118 & 740 cm\(^{-1}\)) in and out of plane of Ar ring.

1HNMR: 2.13 (3H, s, -CH\(_3\)), 6.53 (2H, s, -NH\(_2\)), 7.38 (2H, m, Ar C-H), 7.61 (1H, m, Ar C-H), 7.76 (1H, m, Ar C-H), 8.32 (1H, m, Ar C-H), 9.53 (1H, s, -NH-NH\(_2\)).

Synthesis of final compounds (II a- II d)

A mixture of compounds I (2.45 g, 0.01 mol), one of following phenacyl bromide or its derivatives: phenacyl bromide (1.99 g, 0.01 mol) for compound (II a), p-bromo phenacyl bromide (2.79 g, 0.01 mol) for compound (II b), p-chloro phenacyl bromid (2.33 g, 0.01 mol) for compound (II c), p-nitro phenacyl bromid (2.44 g, 0.01 mol) for compound (II d), and anhydrous sodium acetate (0.82 g, 0.01 mol) in (30 mL) absolute ethanol, was refluxed for 6–10 hrs. The mixture was filtered after cooling, the filtrate was evaporated, the remaining powder washed with water, and dried. The final compounds were isolated and purified by column chromatography by using (silica gel) as stationary phase and (n-hexane with ethyl acetate 2:1) as mobile phase.

Then crystallized from ethanol to give compounds (III a-d) (14, 17).

(II a) 3-((E)-1-(((E)-4-phenyloxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one yellow powder, yield = 50%, M.P. = (40-43) ºC, Rf = 0.73 (A), IR: (3066 cm\(^{-1}\)) N-H stretching of 2º amine, (3016 cm\(^{-1}\)) C-H stretching of Ar ring, (2981 & 2939 cm\(^{-1}\)) C-H stretching of alkane, (1735 cm\(^{-1}\)) C-O stretching of lactone ring, (1693 cm\(^{-1}\)) C=N stretching, (1597 & 1423 cm\(^{-1}\)) C=C stretching of Ar ring (1222, 1242 & 1280 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1076 & 752 cm\(^{-1}\)) in and out of plane of Ar ring.

1HNMR: 2.14 (3H, s, -CH\(_3\)), 5.43 (1H, s, C-NH-C), 7.54 (2H, m, Ar C-H), 7.57 (2H, m, Ar C-H), 7.68 (2H, m, Ar C-H), 7.94 (1H, m, Ar C-H), 7.9 (3H, m, Ar C-H), 7.97 (1H, m, Ar C-H).

(II b) 3-((E)-1-(((E)-4-(4-bromophenyl)oxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one yellow powder, yield = 62%, M.P. = (78-81) ºC, Rf = 0.76 (A), IR: (3089 cm\(^{-1}\)) N-H stretching of 2º amine, (3059 cm\(^{-1}\)) C-H stretching of Ar ring, (2989 & 2951 cm\(^{-1}\)) C-H stretching of alkane, (1743 cm\(^{-1}\)) C-O stretching of lactone ring, (1689 cm\(^{-1}\)) C=N stretching, (1585, 1485 & 1431 cm\(^{-1}\)) C=C stretching of Ar ring (1219 & 1246 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1068 & 813 cm\(^{-1}\)) in and out of plane of Ar ring.

1HNMR: 2.13 (3H, s, -CH\(_3\)), 5.43 (1H, s, C-NH-C), 7.53 (2H, s, -NH\(_2\)), 7.38 (2H, m, Ar C-H), 7.61 (1H, m, Ar C-H), 7.76 (1H, m, Ar C-H), 8.32 (1H, m, Ar C-H), 9.53 (1H, s, -NH-NH\(_2\)).

(II c) 3-((E)-1-(((E)-4-(4-chlorophenyl)oxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one yellow powder, yield = 89%, M.P. = (72-75) ºC, Rf = 0.76 (A), IR: (3069 cm\(^{-1}\)) N-H stretching of 2º amine, (3018 cm\(^{-1}\)) C-H stretching of Ar ring, (2988 & 2951 cm\(^{-1}\)) C-H stretching of alkane, (1735 cm\(^{-1}\)) C-O stretching of lactone ring, (1693 cm\(^{-1}\)) C=N stretching, (1585, 1485 & 1431 cm\(^{-1}\)) C=C stretching of Ar ring (1219 & 1246 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1068 & 813 cm\(^{-1}\)) in and out of plane of Ar ring.

1HNMR: 2.13 (3H, s, -CH\(_3\)), 5.43 (1H, s, C-NH-C), 7.75 (2H, m, Ar C-H), 7.77 (1H, m, Ar C-H), 7.78 (1H, m, Ar C-H), 7.83 (1H, m, Ar C-H), 7.89 (2H, m, Ar C-H), 7.92 (1H, m, Ar C-H).

(II d) 3-((E)-1-(((E)-4-(4-nitrophenyl)oxazol-2(3H)-ylidene)hydrazineylidene)ethyl)-2H-chromen-2-one yellow powder, yield = 76%, M.P. = (78-81) ºC, Rf = 0.76 (A), IR: (3089 cm\(^{-1}\)) N-H stretching of 2º amine, (3059 cm\(^{-1}\)) C-H stretching of Ar ring, (2989 & 2951 cm\(^{-1}\)) C-H stretching of alkane, (1743 cm\(^{-1}\)) C-O stretching of lactone ring, (1689 cm\(^{-1}\)) C-N stretching, (1585, 1485 & 1431 cm\(^{-1}\)) C=C stretching of Ar ring (1219 & 1246 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1068 & 813 cm\(^{-1}\)) in and out of plane of Ar ring.

1HNMR: 2.13 (3H, s, -CH\(_3\)), 5.43 (1H, s, C-NH-C), 7.75 (2H, m, Ar C-H), 7.77 (1H, m, Ar C-H), 7.78 (1H, m, Ar C-H), 7.83 (1H, m, Ar C-H), 7.89 (2H, m, Ar C-H), 7.92 (1H, m, Ar C-H).
Yellowish to orang powder, yield = 65 %, M.P. = (66-69) °C, Rf = 0.76 (A), IR: (3078 cm\(^{-1}\)) N-H stretching of 2º amine, (3020 cm\(^{-1}\)) C-H stretching of Ar ring, (2981 & 2939 cm\(^{-1}\)) C-H stretching of alkane, (1739 cm\(^{-1}\)) C=O stretching of lactone ring, (1693 cm\(^{-1}\)) C=N stretching, (1589, 1489 & 1423 cm\(^{-1}\)) C=C stretching of Ar ring (1219 & 1238 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1083 & 821 cm\(^{-1}\)) in and out of plane of Ar ring. ¹HNMR 2.14 (3H, s, -CH\(_3\)), 5.44 (1H, s, C-NH-C), 7.61 (1H, m, Ar C-H), 7.63 (4H, m, Ar C-H), 7.96 (4H, m, Ar C-H), 7.99 (1H, m, Ar C-H).

Orang powder, yield = 37%, M.P. = (98-101) °C, Rf = 0.68 (A), IR: (3120 cm\(^{-1}\)) N-H stretching of 2º amine, (3082 cm\(^{-1}\)) C-H stretching of Ar ring, (2981 & 2947 cm\(^{-1}\)) C-H stretching of alkane, (1701 cm\(^{-1}\)) C=O stretching of lactone ring, (1600 & 1419 cm\(^{-1}\)) C=C stretching of Ar ring, (1519 & 1346 cm\(^{-1}\)) N-O stretching (asymmetrical and symmetrical), (1215 & 1246 cm\(^{-1}\)) C-O-C stretching of cyclic ether, (1080 & 856 cm\(^{-1}\)) in and out of plane of Ar ring. ¹HNMR 2.15 (3H, s, -CH\(_3\)), 5.52 (1H, s, C-NH-C), 8.18 (2H, m, Ar C-H), 8.19 (3H, m, Ar C-H), 8.35 (3H, m, Ar C-H), 8.37 (2H, m, Ar C-H).

Anti-inflammatory Study

The inflammatory model that used to evaluate final compounds (II a-d) for the in vivo acute anti-inflammatory effects exploited egg-white induced rat paw edema, for comparison with the anti-inflammatory activity of celecoxib. The decrease of paw thickness is the basis of screening of the newly synthesized compounds for their anti-inflammatory activity.

Methods of Anti-inflammatory study

Both sex albino rats (200 ± 10) were provided by Iraqi Center for Cancer & Medical Genetic Research / Al-Mustansiriyah University, and housed under standardized conditions in animal house. Animals were divided into six groups (six rats per group) as follow: Group 1: injected with the vehicle (in DMSO) with a dose of 2 ml/kg and served as a control group. Group 2: injected with celebrax with a dose of 5mg/kg (18), dissolved in DMSO, and served as a reference group. Group 3-6: each group of six rats injected with the tested compounds (III a-d) respectively, in doses (4.5 mg/kg for comp II a, 5.5 mg/kg for comp II b, 5 mg/kg for comp II c, and 5.1 mg/kg for comp II d), dissolved in DMSO. The reference substance celecoxib was administered both with the tested compounds by the intra-peritoneal route (i.p.). Egg albumin was used to induce rat paw edema as an acute inflammatory model for studying the activity of the final compounds. 0.05mL of undiluted ovalbumin was subcutaneously injected into the left hand paw of the rats; preceded by a half hour of intraperitoneal injection of the drugs or their vehicle (19). Digital vernier used for measuring paw thickness at 7 periods (0, 30, 60, 120, 180, 240, and 300 minutes) and these measurements were taken after the intra-peritoneal administration of the tested compounds or DMSO (control), considered as time zero (20).

In this study there was no significant difference among the tested compounds compared to celecoxib and control at baseline, after 30 and 60 minutes. However, after 120 to 300 minutes, compounds (II b, II c, and II d) showed more activities to reduce paw thickness compared to celecoxib, in which (III d) showed best reduction in paw thickness compared to the other compounds. Compounds (II a) showed less activities to reduce paw thickness when compared to the standard celecoxib after. Among them, compounds (II b, II c, and II d) was found to exhibit the best reduction in paw
thickness relative to the other compounds as illustrated in Figure (2) and Table (1).

Table (1): Effect of Dimethyl sulfoxide (control), celecoxib (standard) and target compounds (II a-II d) on induced paw edema

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Standard</th>
<th>II a</th>
<th>II b</th>
<th>II c</th>
<th>II d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.70±0.03a</td>
<td>3.51±0.03ab</td>
<td>3.63±0.06ab</td>
<td>3.53±0.16ab</td>
<td>3.46±0.04ab</td>
<td>3.40±0.07b</td>
</tr>
<tr>
<td>30</td>
<td>5.56±0.03ab</td>
<td>5.50±0.04ab</td>
<td>5.48±0.08d</td>
<td>5.33±0.11bc</td>
<td>5.31±0.04bc</td>
<td>5.10±0.03c</td>
</tr>
<tr>
<td>60</td>
<td>6.6±0.06b</td>
<td>6.11±0.06b</td>
<td>6.55±0.05a</td>
<td>5.96±0.08b</td>
<td>5.83±0.03d</td>
<td>5.86±0.04e</td>
</tr>
<tr>
<td>120</td>
<td>6.95±0.06a</td>
<td>5.11±0.07d</td>
<td>6.08±0.06d</td>
<td>4.75±0.08e</td>
<td>4.50±0.04f</td>
<td>4.41±0.07f</td>
</tr>
<tr>
<td>180</td>
<td>6.08±0.06a</td>
<td>4.86±0.08c</td>
<td>5.11±0.07d</td>
<td>4.35±0.10e</td>
<td>4.35±0.05e</td>
<td>3.98±0.04f</td>
</tr>
<tr>
<td>240</td>
<td>5.76±0.06a</td>
<td>4.55±0.07b</td>
<td>4.66±0.06c</td>
<td>3.80±0.11e</td>
<td>4.10±0.05d</td>
<td>3.76±0.07e</td>
</tr>
<tr>
<td>300</td>
<td>5.48±0.07a</td>
<td>4.21±0.07b</td>
<td>4.40±0.04b</td>
<td>3.55±0.09d</td>
<td>3.80±0.06c</td>
<td>3.70±0.06cd</td>
</tr>
</tbody>
</table>

Data are expressed in mm paw thickness as mean ± SEM.

Means with a different letter in the same row are significantly different (P<0.05)

Figure (2): Effect of celecoxib (reference), DMSO (control), and compounds III a-III f on induced paw edema
edema in rats.

**Conclusion**

The proposed compounds were successfully synthesized, and their chemical structures and purity were determined using IR spectroscopy and ^1^HNMR. The anti-inflammatory was tested using control (solvent DMSO) and standards (celecoxib). The compounds (II b, II c, and II d) showed good anti-inflammatory activity, when compared with standard compound.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


The Sensitivity of some Types of Citrus Fruits, the role of the Propolis and the Color of Storage Bags in the Development of Green Mold Caused by Fungi *Penicillium Digitatum*

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¹Assistant Lecturer, ²Lecturer College of Agriculture/ University of Anbar/Iraq

Abstract

The results of the study indicated that green mold caused by *Penicillium digitatum* was noticed in all types of citrus fruits (orange, mandarin, bitter orange, lemon). The most sensitive among these fruits was the mandarin with 56.12% infection, followed by orange with 40.18%. On the other hand, the bitter orange and the lemon showed more resistant to the disease, and the their infection percentages were (32.14% and 30.55% respectively. The results indicated that the color of the bags had a great effect on the development of the disease where the infection percentages when using light green bags and light yellow bags were (19.68%, 17.24%) respectively. While for the dark black bags, the infection percentage was 13.73%. The use of porpolis in all concentrations (6, 8, 10, 12) g/l did not lessen the size of the infected area compared with contaminated treatment by pathogen.

**Keywords:** food; nutrition; toxicity; disease; humidity

**Introduction**

Citrus crops suffer from many diseases, whether before or after the harvest, which cause losses in the quantity and quality of production. Perhaps the most important of these diseases are fungal diseases, especially the green mold disease, as the losses resulting from this disease in 2017 were estimated at 50 million metric tons in cultivation areas of all citrus fruits (15). This disease is spread in most citrus cultivation areas in the world, especially in warm areas with high humidity (1,2,3). The risk this disease can cause increases when appropriate conditions are available such as a temperature ranging between 5 - 25 ° C and relative humidity of more than 80% with the availability of lighting required for the production of spores (4,5). Scratches formed during harvesting and the presence of insects also help in the development of the disease. Moreover, *P. digitatium* has the ability of producing some cellulose and pectin enzymes that increase the risk of this disease (6). Several methods were used to fight this disease, such as the use of paraffin wax, evaporation inhibitors (7) and chemical pesticides (8). In order to know the importance causes of the disease, its severity on citrus fruits and how to reduce it, the study was conducted according to the following axes:

1- Investigating the sensitivity of several citrus to the infection of the green mold disease.

2-The role of storage bags and role of propolis in the development of the disease.

**Materials and Methods**

This experiment was carried out at the University Of Anbar/ College of Agriculture/ Plant Protection Department/ Plant Pathology Laboratory for the winter season 2017-2018. Fruits of different types of citrus were collected (orange, bitter orange, lemon, and mandarine) from the orchards of the district of Heet, Ramadi and Fallujah. These fruits showed symptoms of infection with the disease (9). These infected fruits were placed in sterile polyethylene bags and brought to the laboratory, then pieces from the infected area were extracted from the fruits located in the collection areas. The pieces
taken were 1 cm in length. They were later placed in petri dishes containing PDA media. They were later transferred to the incubator at a temperature of 25 °C for a period of 7 days. After that, the Pathogenic fungus were identified according to the species key (10), and the pathogen was diagnosed to the type level depending on the type key (11).

**Sporophobia Preparation**

Sporophytes were prepared according to (12), 1 ml sterile distilled water was added to each growing petri dish in which the *Penicillium digitatum* was observed, and the water was moved stirred the plate by using a sterile L-shaped glass rod. Then the water containing the sporophyte of the fungus was extracted and put into a test tube A 9 ml container of sterile distilled water. After that, a series of dilutions were added until the concentration reached is 10-7 spores/ml. This concentration is determined using a Haemocytometer slice.

**The sensitivity of some Citrus Fruits to Green Mold Caused by the Fungus Penicillium digitatum**

This experiment was carried out according to the randomized complete block design (RCBD). The treatments were divided as follows

1. T1 = treatment of orange fruit
2. T2 = treatment of mandarin fruits
3. T3 = treatment of (bitter orange ) fruits
4. T4 = treatment of lemon fruits
5. T5 = contaminated pathogen treatment
6. T6 = sound comparison treatment

Each treatment included 4 replicates. In each iteration, there were 3 healthy homogeneous fruits from all types of citrus used. Then all fruits were scratched with superficial scratches orthogonally by a sterile knife of 1 cm length, then all treatments were contaminated, except for the T6 treatment, with the pathogenic fungus of the concentration of 10-7 spores/ml, and then the fruits of each iteration were placed in a sterile polyethylene bag perforated by a cork drill 5 ml. Then, it was left under the natural conditions of the laboratory. Measurements were taken after (10) days by measuring the rate of fungal growth of the infected spot, and then calculating the percentage of the infected area according to the formula:

\[
\% \text{ infected area} = \frac{\text{Fung growth rate}}{\text{The peripheral growth rate of the fruit}} \times 100.
\]

The effect of Citrus Storage Bags Color on the Development of Green Mold Caused by Fungus Penicillium Digitatum

In this experiment, orange fruits, bitter orange, lemon, and local mandarin were used as homogeneous healthy fruits were selected. The treatments were distributed according to RCBD design. The treatments were conducted as follows:

1. T1 = dark black storage bags
2. T2 = Transparent green storage bags
3. T3 = transparent yellow storage bags
4. T4 = exposed orange fruits contaminated with the pathogen
5. T5 = healthy exposed orange fruits

Four replicates were used for each treatment at the rate of 3 orange fruits per replicate. These fruits were scratched superficially, as explained in the previous paragraph. After that all the treatments were contaminated, except for the T5 treatment, by spore concentration at 10-7 spores/ ml, then the bags were sealed tightly and left under laboratory conditions for 10 days. Then the fungal growth of the infected area caused by the fungus was measured. The Effect of Different Propolis Concentrations on % of the *P. digitatum* Infection Area

Propolis was obtained from the apiary section of the Plant Protection Department/ College of Agriculture/ University Of Anbar, then (6, 8, 10, 12) grams of propolis were weighed. Then each weight was placed in a glass beaker containing 1000 mL sterile distilled water. Then the water was heated to a temperature of 60-70 °C with constant stirring for 5 minutes. The resulting solution was later sprayed on the scratched orange fruits, and then the fungus *P. digitatum* was sprayed at a concentration of 10-7 spores/ ml. The same process was repeated for all weights of the propolis In the same
way. Three replications were used for each treatment and three oranges for each iteration. The contaminated comparison treatment was treated with the same fungus solution on the scratched fruits. The treated fruits were left for 10 days under laboratory conditions, after which the fungal growth rate of the infected area was measured for each treatment, and the percentage of the infected area was calculated according to the following formula:

\[
\text{% for the infected area} = \frac{\text{Fung growth rate}}{\text{The peripheral growth rate of the orange}} \times 100
\]

Results and Discussion

Table (1) shows that green mold disease caused by \textit{P. digitatum} is spread in all citrus cultivation areas and in all types of citrus fruits used in this study, and this confirms what is reported in \textsuperscript{(13, 14)}, that this disease is spread in most areas of citrus cultivation in the world. The table also shows that the severity of the disease increases on the fruits of these citrus after the harvest \textsuperscript{(15, 16)}. The risk of this disease is found to be due to the ability of the pathogen (\textit{P. digitatum}) to grow in a temperature range of 5-25 °C when moisture is available at a rate ranging from 75 - 80% \textsuperscript{(1)}.

Table (2) shows that the fruits of the citrus fruits used (orange, bitter orange, lemon, mandarin) varied in their sensitivity to green mold disease, as mandarin fruits recorded the highest levels of sensitivity, and the percentage of infection area reached 56.12%, followed by orange fruits with a percentage of 40.18%, while the fruits of lemon and bitter orange were less sensitive to green mold, as the percentage of the infection area was (30.55%, 32.14%) respectively. This difference in the sensitivity of the citrus fruits used may be due to the difference in the thickness of the outer wall of the fruits and the difference in the cellular composition of the cell wall of these fruits, especially in the difference in the concentration of ethylene gas of the most important means of defense in citrus fruits \textsuperscript{(17,18)}.

Table (3) shows that the color of the storage bags used in preserving citrus fruits had an effect on the development of infection with green mold caused by the fungus \textit{P. digitatum}. Particularly, transparent colors such as transparent yellow and transparent green recorded an infection rate of 17.24% and 19.68%, respectively, while the dark black color was the least affected in the development of the disease, as the percentage of the infected area was 13.73%. This effect is ascribed to the color of the storage bags which affects the amount of light passing through them, which is a key factor in the formation of the pathogen spores. The table also shows that the overlap between the fruits of different types of citrus fruits and the color of the storage bags has a significant impact on the development of the disease. As for the fruits of bitter orange placed in dark bags, it was the lowest in the infection rate with 2.38%. As illustrated in Table (4), the use of the propolis did not lead to any significant reduction in the infected area, and the reason may be attributed to the fact that the heat used led to the decomposition of the substances has a role in resisting the disease.

Table (1) Results of isolating \textit{P. digitatum} from different citrus fruits in some areas of Al Anbar Governorate

<table>
<thead>
<tr>
<th>Fruits Collection Areas</th>
<th>Type of Fung Isolated from the infected fruits</th>
<th>Fruit Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramadi</td>
<td>P. digitatum</td>
<td>Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Bitter Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Mandarin</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Lemon</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Bitter Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Mandarin</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Lemon</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Bitter Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Mandarin</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Lemon</td>
</tr>
</tbody>
</table>

Table (2) Results of isolating \textit{P. digitatum} from different citrus fruits in some areas of Al Anbar Governorate

<table>
<thead>
<tr>
<th>Fruits Collection Areas</th>
<th>Type of Fung Isolated from the infected fruits</th>
<th>Fruit Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramadi</td>
<td>P. digitatum</td>
<td>Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Bitter Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Mandarin</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Lemon</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Bitter Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Mandarin</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Lemon</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Bitter Orange</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Mandarin</td>
</tr>
<tr>
<td></td>
<td>P. digitatum</td>
<td>Lemon</td>
</tr>
</tbody>
</table>
Table (2) the sensitivity of some types of citrus fruits to green mold disease caused by *P. digitatum* under laboratory conditions

<table>
<thead>
<tr>
<th>% Infected Area</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 D</td>
<td>A1B1</td>
</tr>
<tr>
<td>56.12 A</td>
<td>A1B2</td>
</tr>
<tr>
<td>0.00 D</td>
<td>A2B1</td>
</tr>
<tr>
<td>40.18 B</td>
<td>A2B2</td>
</tr>
<tr>
<td>0.00 D</td>
<td>A3B1</td>
</tr>
<tr>
<td>32.14 C</td>
<td>A3B2</td>
</tr>
<tr>
<td>0.00 D</td>
<td>A4B1</td>
</tr>
<tr>
<td>30.55 C</td>
<td>A4B2</td>
</tr>
</tbody>
</table>


** Each number represents an average of four iterations and in each iteration there are three fruits.

***Treatments that share the same letter show no significant differences among them according to the choice of Dunkin polynomial at the level of significance 5%.

Table (3) the effect of interference between different types of citrus fruits and the color of storage bags on the development of green mold disease (*P. digitatum*) under laboratory conditions

<table>
<thead>
<tr>
<th>% The infection with the disease in different citrus types</th>
<th>effect of interference between different types of citrus fruits and the color of storage bags on the development of green mold disease</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B5</td>
<td>B4</td>
</tr>
<tr>
<td>A 16.49</td>
<td>0.00 H</td>
<td>11.92 EF</td>
</tr>
<tr>
<td>A 16.25</td>
<td>0.00 H</td>
<td>14.08 DE</td>
</tr>
<tr>
<td>B 3.33</td>
<td>0.00 H</td>
<td>2.38 GH</td>
</tr>
<tr>
<td></td>
<td>0.00 D</td>
<td>9.45 C</td>
</tr>
</tbody>
</table>

* A1 = orange fruits, A2= mandarin fruits, A3= bitter orange fruits, B1= dark black, B2= transparent green, B3= transparent yellow, B4= contamination-free treatment without bags, B5= healthy comparison treatment without bags.
** Each number represents an average of four iterations and in each iteration there are three fruits.

*** Treatments that share the same letter show no significant differences between them, according to the choice of Dunkin polynomial at the level of significance 5%

Table (4) the effect of different concentrations of propolis on fruits’ resistance against green mold disease caused by *P. digitatium*

<table>
<thead>
<tr>
<th>% for the infected area</th>
<th>Equation Symbol</th>
<th>The Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.66 %A</td>
<td>T1</td>
<td>Orange Fruits</td>
</tr>
<tr>
<td>18.30 %A</td>
<td>T2</td>
<td>Orange Fruits</td>
</tr>
<tr>
<td>18.15 %A</td>
<td>T3</td>
<td>Orange Fruits</td>
</tr>
<tr>
<td>18 %A</td>
<td>T4</td>
<td>Orange Fruits</td>
</tr>
<tr>
<td>18.80 %A</td>
<td>T5</td>
<td>Orange Fruits</td>
</tr>
</tbody>
</table>

* A1= Concentration of 6g / L, A2= 8g / L, A3= 10g / L, T4= 12g / L T5= contaminated treatment with fungus *P. digitatium*

** 3 replicates were used for each treatment with 3 fruits in each replicate

*** Results were analyzed according to Dunkin polynomial results under the probability of 5%.

*** Treatments that share the same letter show no significant differences among them.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


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15. Smilanick J.L, Mansour M.F. and Sorenson D. Pre- and postharvest treatments to control Green Mold of citrus fruit During Ethylene Degreening. Plant. Dis. 2006; (90) 89-96.


The response of Brahimi apple’s Cultivar to Foliar Application of Glycyrrhizin Extract (GLE) and Humic Acid (HA)

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Abstract

The research team had conducted this work at a private orchard in Saqlawiya – Falluja district in 2019 to investigate the influence of foliar application of liquorice (glycyrrhizin) extract (GLE) and humic acid (HA) on some vegetative growth traits and yield of Al-Ibrahimi apple cultivar. The factorial experiment consisted of 2 factors; the first one was GLE which consisted of 3 levels (L0, L1, L2) of (0, 3, and 6 g.L⁻¹) respectively; while the second one was HA with 4 levels (0, 2, 4, and 6 g.L⁻¹) had symbols of (H0, H1, H2, and H3) successively. Thus, the total number of treatments was 12 of 3 replications. Every apple tree considered as an experimental unit. The HA treatments were applied 7 times to soil every 20 days starting from 20th February 2019 while the foliar applications of GLE were 5 times starting from the 1st April in the same year.

A significant improvement in vegetative growth, quantity, and quality of yield due to GLE and HA applications has been shown in increasing individual leaf area (LA), length of branches (LOB), number of leaves (NOL), total chlorophyll content in leaves (TCL), and total carbohydrates content in leaves (TCCL), average weight of fruits (AWF), and number of fruits per tree (NFT) that reflected positively on increasing yield per individual tree (YPT), total percentage of soluble solids (PSS), vitamin C content in fruits (CC), and decreasing acidity of fruits (TPA). The interaction between factors had significant influence in most of studied traits. Thus the L1H3, L2H2, and L2H3 treatment had the best results.

Keywords: Liquorice; Humic acid; Foliar application; Fruits; Apple; toxicity

Introduction

The apple (Malus pumila L.) is one of Rosaceae family which is the most worldwide spread type of deciduous fruit trees in temperate regions in the globe, the global production of apples is estimated at 83139326 tons, and Iraq’s production of apples is estimated at 626,470 tons(1). Many scientific manuscripts had indicated that the foliar spraying of many herbal extracts had a great effect in improving the vegetative and flowering growth and yield of many plants(2). Among these plant extracts is the extract of licorice plant roots, Glycyrrhiza glabrag, which has been proven in many studies to enhance the productivity of many plants (3), (4) and (5). Found that in spraying of pear seedlings with licorice root extract, had significant increase in plant height, stem diameter, dry weight of leaves and leaf content of chlorophyll. Found that spraying of pear seedlings with licorice root extract has led to significant increase in the leaf area and its total chlorophyll content. (6) Stated an increase in the yield of pomegranate trees when spraying leaves with licorice root extract. The use of organic products has received a lot of attention in the recent period, furthermore, this new system of agricultural production that contain Humic acid has become one of the most important organic compounds used in this field, if it is found that the addition of humic acid to soil leads to increase the cation exchange capacity of soil and increases its pH and thus increases the availability of nutrients and increases

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the growth of roots and their branches (7), (8) found in their study on Anna cultivar apples that the addition of humic acid to soil by 30 cm\(^3\).tree\(^{-1}\) has led to significant increase in the number of leaves per branch\(^{-1}\) and the area of the leaf increased with an increase in yield by 25.6% in addition to an increase in the average weight of the fruit and the percentage of total soluble solids, (9) also obtained similar results when studying Anna-cultivar apple trees, and (10) stated an increase in tree yield, fruit count per tree, and average of fruit weight of pear (Leconte cultivar) when adding 100 ml per tree of humate-NPK combination (10% humate ratio) to the soil. (11) Had gained the same results on apricot trees (Canino cultivar).

**Materials and Methods**

The effect of foliar spraying with glycyrrhizin root extract (GLE) and the addition of humic acid (HA) to the soil has been studied on some of the vegetative growth characteristics and yield of apple trees (Brahimi cultivar). The factorial experiment of randomized completely block design (RCBD) has been conducted where the GLE was the 1\(^{st}\) factor with levels of 0, 3, 6 g.L\(^{-1}\) have symbols of L0, L1, and L2 respectively, whereas the 2\(^{nd}\) factor were HA levels of 0, 2, 4, 6 g. L\(^{-1}\) have the symbols of H0, H1, H2, H3 for the Humibest product from Meristem Spanish company (have 55% Humic acid, 5% Fulvic acid, and 7% K\(_2\)O) as a humic source. The required quantity of HA was dissolved in 15 liters of water and added circularly around each tree on 50 cm distance and 20 cm depth from main stem, then the HA was applied to soil starting from 20\(^{th}\) February for every 20 days at 7 times. The trees were sprayed with GLE five times every 20 days starting from 1\(^{st}\) April 2019, the results were analyzed using GenStat and then, averages were compared using the least significant difference test (L.S.D) at a probability level of 5%. The following traits have been studied:

1- Individual leaf area (cm\(^2\)) (LA):

The results of table 1. Showed that the individual LA had significantly affected by the increased levels of foliar sprayed GLE. The highest LA average was 21.23 cm\(^2\) for L2 level compared to 19.47 cm\(^2\) in control treatment. The same table revealed that H3 level of HA led to significant increase in individual LA, where it was 21.02 cm\(^2\) compared to insignificant difference of H1 and H2 levels that reached 20.35 and 20.05 cm\(^2\) respectively as well as that H0 had less value of 19.40 cm\(^2\). On other hand, the interaction between GLE and HA had insignificant effect on individual LA.
Table 1. The effect of foliar GLE, soil applied HA, and their interaction on individual LA (cm²) for Brahimi apple in spring season of 2019

<table>
<thead>
<tr>
<th>HA GLE</th>
<th>H0</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>Average GLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L0</td>
<td>18.62</td>
<td>19.15</td>
<td>19.71</td>
<td>20.38</td>
<td>19.47</td>
</tr>
<tr>
<td>L1</td>
<td>19.43</td>
<td>19.86</td>
<td>20.06</td>
<td>20.36</td>
<td>19.93</td>
</tr>
<tr>
<td>Average of HA</td>
<td>19.40</td>
<td>20.05</td>
<td>20.35</td>
<td>21.02</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D %5 | HA GLE interaction
---------|---------------------
1.13     | 0.98                insignificant

2. Increase average of branch length (LOB) (cm):

Table 2 had shown a significant increase in average LOB for every single increase in foliar GLE levels. The highest LOB value of 30.98 cm was found in L2 compared to 24.04 cm in control treatment.

The same table had revealed significant differences among HA levels that affected LOB average. The highest LOB value was 30.33 cm found in H3 compared to 24.22 cm in control treatment H0 which was the least.

The interactions between GLE and HA treatments were significant in increasing LOB values where the highest averages of 33.33, 30.5, 31.33, and 32.42 cm were found in L1H3, L2H1, L2H2, and L2H3 interactions respectively compared to 19.25 cm in control treatment.

**Table 2. The effect of foliar GLE, soil applied HA, and their interaction on LOB (cm) for Brahimi apple in spring season of 2019.**

<table>
<thead>
<tr>
<th>HA GLE</th>
<th>H0</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>Average of GLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L0</td>
<td>19.25</td>
<td>24.25</td>
<td>27.42</td>
<td>25.25</td>
<td>24.04</td>
</tr>
<tr>
<td>L1</td>
<td>23.75</td>
<td>27.08</td>
<td>27.33</td>
<td>33.33</td>
<td>27.88</td>
</tr>
<tr>
<td>L2</td>
<td>29.67</td>
<td>30.5</td>
<td>31.33</td>
<td>32.42</td>
<td>30.98</td>
</tr>
<tr>
<td>Average of HA</td>
<td>24.22</td>
<td>27.28</td>
<td>28.69</td>
<td>30.33</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D %5 | HA GLE interactions
---------|---------------------
1.85     | 1.61                3.21
3. Increase average of number of leaves per branch NOL (leaf per branch LPB):

The results of table 3 revealed significant differences among levels of foliar GLE in increasing average NOL. The second level L2 had the highest NOL of 22.51 LPB and insignificant NOL difference of 21.50 LPB in L1 compared to control treatment which had the least average of 16.16 LPB. The results of the same table showed that NOL was significantly increased with HA levels where the highest was 25.28 LPB in H3 compared to the least NOL in H0 which was 15.56LPB.

There was a significant effect of interactions between GLE and HA on increasing NOL values where the highest of them found in L1H3, L2H2, and L2H3 treatments of (30.17, 28.63, and 25.92 LPB) respectively with insignificant differences among them compared to the least of 14.25 LPB in control treatment.

Table 3. The effect of foliar GLE, soil applied HA, and their interaction on NOL (LPB) for Brahimi apple in spring season of 2019.

<table>
<thead>
<tr>
<th>HA GLE</th>
<th>H0</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>Average of GLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L0</td>
<td>14.25</td>
<td>17.22</td>
<td>13.42</td>
<td>19.75</td>
<td>16.16</td>
</tr>
<tr>
<td>L1</td>
<td>15.50</td>
<td>19.08</td>
<td>21.25</td>
<td>30.17</td>
<td>21.50</td>
</tr>
<tr>
<td>L2</td>
<td>16.92</td>
<td>18.58</td>
<td>28.63</td>
<td>25.92</td>
<td>22.51</td>
</tr>
<tr>
<td>Average of HA</td>
<td>15.56</td>
<td>18.29</td>
<td>21.10</td>
<td>25.28</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D %5

<table>
<thead>
<tr>
<th>HA GLE</th>
<th>HA</th>
<th>GLE</th>
<th>interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.47</td>
<td>2.14</td>
<td>4.28</td>
</tr>
</tbody>
</table>

The increase in vegetative growth traits, consisting of LA, LOB, and NOL when spraying with GLE may be due to the fact that this extract contains a good amount of nutrients necessary for plant growth such as potassium, phosphorus, calcium, other elements, various amino acids, salts and carbohydrates (appendix 1) which are included in the synthesis of many enzymes, especially in the enzymes contained in photosynthesis, as well as the providing of nitrogen plant directly at the spraying (14). The role of GL extract in improving the growth of the plant may be due to its role similar to the work of gibberellin, since it contains mevalonic acid, the bio-initiator of the formation of gibberellin. It is known that the gibberellins work to increase the softness of the cellular walls of the top and sub-top symbiotic, which increases from its permeability and allowing the entry of larger amounts of water and nutrients, which increases the growth and expansion of cells and this is reflected in the elongation of plant branches and increase the leaf area and the gibberellins also work to stimulate the production of auxins and reduce their degradation (15).

(16) Got a significant increase on branches’ length, leaves number, and leaf area of Canino cultivar apricot trees.

4. Total content of chlorophyll in leaves (mg.100 g⁻¹ fresh weight) TCL:

The results of table 4 referred to significant increase of TCL with increasing levels of foliar GLE. The second level L2 has the highest value of 114.0 mg.100 g⁻¹ fresh weight in comparison with least value of 107.6 mg.100 g⁻¹ fresh weight in control treatment. A significant increase of TCL noticed from the same table as a result of increasing HA levels in soil where the highest value of 118.6 mg.100 g⁻¹ fresh weight was shown in H3 treatment compared to control treatment which had the least TCL of 104.7 mg.100 g⁻¹ fresh weight. The interaction between GLE and HA treatments had a powerful effect in increasing TCL where the treatment
L2H3 had the highest value of 122.7 mg.100 g\(^{-1}\) fresh and the lowest value of 102.3 mg.100 g\(^{-1}\) fresh weight was recorded in L0H0 treatment.

Table 4. The effect of foliar GLE, soil applied HA , and their interaction on TCL (mg.100 g\(^{-1}\) fresh weight) 
for Brahimi apple in spring season of 2019.

<table>
<thead>
<tr>
<th>HA GLE</th>
<th>H0</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>Average of GLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L0</td>
<td>102.3</td>
<td>104.0</td>
<td>109.0</td>
<td>115.0</td>
<td>107.6</td>
</tr>
<tr>
<td>L1</td>
<td>105.0</td>
<td>105.3</td>
<td>110.3</td>
<td>118.0</td>
<td>109.7</td>
</tr>
<tr>
<td>L2</td>
<td>106.7</td>
<td>107.3</td>
<td>119.3</td>
<td>122.7</td>
<td>114.0</td>
</tr>
<tr>
<td>Average of HA</td>
<td>104.7</td>
<td>105.6</td>
<td>112.9</td>
<td>118.6</td>
<td></td>
</tr>
<tr>
<td>L.S.D %5</td>
<td>1.1</td>
<td>0.9</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The increase in TCL leaves when spraying leaves with GLE may be due to the growth stimulators like macro and micro nutrients that this extract contains, especially nitrogen, which is included in the porphyrin ring, which is the basis for the synthesis of chlorophyll, since 70% of the leaf nitrogen, is included in the formulation of this formula.

**Conclusions**

1- The 6 of GLE and the addition of HA to the soil with levels of 4 and 6 gram per liter had a significant effect in most of the studied traits.

2- The interaction between the two study factors had a significant effect on most of the studied traits, and the best results were in the L2H2 and L2H3 interaction treatments.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**

1. EL-Sehrawy O. M. . Reducing the amount of nitrile and nitrile in Anna apple Fruits by using inorganic N along with EM , yeasf and humic acid . Alexandrina science Exchange J. 2015;36 (2) 188 – 196 .


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A Comparative Study of Cognitive Achievement in Football Among Students who Have Different Learning Styles

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Abstract
The study aims at identifying the differences in cognitive achievement in football among students with different learning styles. The researchers used the descriptive approach with a comparative study method for its suitability for the nature of the problem, and the sample of the examined students who are the fourth stage students in the Faculty of Physical Education and Sports Science - Samarra University for the academic year 2018-2019. The number of students is (108), and the researchers used the Learning Styles Scale (VAK) that was developed at the Qattan Center for Educational Research and Development and a measure of cognitive achievement in football. The main experiment was carried out on Sunday 5/5/2019 to statistically manipulate data, the researchers used the Statistical Package (SPSS) to find: (mean, standard deviation, simple correlation coefficient Pearson, F test for variance analysis, and L.S.D test). The researchers concluded that there were significant differences in cognitive achievement in football among students with different learning styles. students who preferred visual learning style over students who preferred auditory and kinesthetic learning styles, and students who preferred auditory learning style superior to students who preferred learning style Sens - kinesthetic.

Keywords: Cognitive achievement, football, learning styles (VAK).

Introduction
The learners at any school grade have a set of characteristics, characteristics, and criteria that make him different from other students, and he has the independent personality that leads to a clear difference between him and his colleagues in his view of learning, and the acquisition of different concepts and skills. that helps him to meet his needs, desires, and needs. his motivation to learn increased and his knowledge acquisition increased. Moreover, from the premise that the learning and teaching process is a very complicated issue in terms of the process of its occurrence, especially if we realize that each learner has his method of learning and acquiring concepts through the experiences that he is going through and how he deals with it, i.e. the preferred educational style through which the learner learns. The need to understand the Styles of learning (VAK) is increasing according to the need of group learning within heterogeneous classes and educational literature has given this aspect attention, as the basis of research, in general, must be the learner with all his dimensions as the basic learning unit (1).

The learning style of a particular student includes his preferred method of receiving information and how to process it. Some learners prefer to hear the educational material with the method of lecture, and the other section prefers displaying the educational material by pictures, slides, and video. There is another section that prefers movement during the learning process and direct participation in it to learn (2). Some people prefer to learn in a group while others prefer to work alone, as some prefer to know the subject in a general way first and then the details and some favor the opposite. (3)

Procedures and measurements
Participants
The examined students were chosen from the fourth...
stage students in the Faculty of Physical Education and Sports Science - Samarra University for the academic year 2019-2020 and they are (115) students, by defining the learning style using the scale of preferred learning styles. It was found that the sample prefers three modes of learning which are the learning style Sense, kinesthetic and visual learning style, and auditory learning style. Accordingly, the sample of (108) students was divided into three groups, the first group that preferred the kinesthetic style consisted of (32) students and the second group that preferred the visual learning style consisted of (40) students. The third group that avoids the auditory learning pattern is composed of (36) students, whose percentage is (93.9%) of the total research community.

**Procedures:**

**Learning Style Scale (VAK):** To achieve the aims of the research, the researchers used the preferred Learning Styles Scale (VAK) which was developed at the Qattan Center for Educational Research and Development (1), which is consisted of (80) A paragraph divided into three dimensions:

- The first dimension (20) Items which represent the kinetic learning style, which is from (1-20).

  The second dimension (40) items represent the visual-verbal and non-verbal learning style, which is from (21-60).

  The third dimension (20) is an item that represents the auditory learning style, and it is from (61-80).

The results of the feedback to the scale are calculated to determine the preferred pattern by giving (4) degrees to the answer (always), (3) degrees to the answer (often), two degrees to the answer (sometimes), and one degree to the answer (never), and if you get a high degree in one of the three styles (sensation - kinesthetic, visual, auditory), this indicates that you prefer this style in learning situations.

The used scale gained validity after presenting it in its initial form to a group of experts in the field of educational psychology, physical education, and sports science.

Then stability of the scale was found using the Retest method (4) (5) (6), as the test was applied to a sample of students outside the final application sample and within community research, and then re-application of the scale after two weeks from the first application. Then, after collecting the scale forms were statistically processed using the simple correlation coefficient (Pearson) between the degrees of the first and second application, so the coefficient of stability of the sense-kinematic style (0.82), and the visual style (0.76), it has high stability that can be used in the application of the instrument. The higher the stability coefficient value (0.71), the better. (7)

**Football Cognitive Achievement Scale:**

To measure cognitive achievement of basic football skills, the two researchers adopted the test designed by (4) and is specifically designed to measure cognitive achievement of basic football skills. This test uses objective questions based on multiple-choice, as it contains (40) patterns and each pattern contains (4) alternatives that achieve the goals related to the educational content of basic skills in football. one score is calculated for each correct answer. To find the scientific foundations of the scale, the researchers followed the same previous procedures where the validity of the scale was extracted by presenting it in its initial form. Also, it included (40) patterns on the experience and specialization in the field of football, and the opinions of the arbitrators were taken as (5) patterns (5, 9, 17, 22, 34) were deleted. Thus, in its final form, it contains (35) paragraphs. According to the arbitrators, it is suitable for students of the fourth stage in the College of Physical Education and Sports Science, and thus the scale has obtained apparent honesty.

To ensure the appropriateness of the scale, and to find consistency for it, a survey which is consisted of (4) students is applied. This correlation has reached (0.87) and this stability is valid for the study.

The participants of the survey answered the cognitive achievement scale in football on Sunday (5/5/2019), and the number (108) students were divided into three groups according to their preferred educational items, and without specifying a time to answer the scale, and the correct answer method was explained on the scale, and that By putting a circle around the correct answer that the student thinks appropriate, and after completing the answer to all the items, the scale questionnaires were
collected from them, and thus the final exam score is the sum of his correct answers on the scale.

**Data analysis:**

The data is prepared, organized, and entered into the computer as an XL file; Various statistical methods were used to analyze and process statistical data using SPSS V27, then the standard deviation, then the simple correlation coefficient Pearson, the F test for variance analysis as well as the least significant mean difference of L.S.D were used.

**Results**

**Table 1: Shows the statistical variables related to cognitive achievement according to preferred learning styles used in this study**

<table>
<thead>
<tr>
<th>Items</th>
<th>Measuring unit</th>
<th>Arithmetic mean (s-)</th>
<th>standard deviation (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual learning Item</td>
<td>score</td>
<td>29.083</td>
<td>2.151</td>
</tr>
<tr>
<td>Auditory learning Item</td>
<td>score</td>
<td>22.5</td>
<td>1.90</td>
</tr>
<tr>
<td>Kinesthetic learning Item</td>
<td>score</td>
<td>17.666</td>
<td>2.549</td>
</tr>
</tbody>
</table>

The arithmetic mean of the visual learning item was (29.083), with a standard deviation of (2.151).

- The arithmetic means for the audio learning item was (22.5) with a standard deviation of (1.90).

- The arithmetic means for the kinetic learning item was (17.666) with a standard deviation of (2.549).

**Table 2: shows the results of the test (F) between the three learning styles in the cognitive achievement test for football**

<table>
<thead>
<tr>
<th>variable</th>
<th>Measurement unit</th>
<th>Source of divergence</th>
<th>Total squares</th>
<th>Degree of freedom</th>
<th>Average of squares</th>
<th>value(F)</th>
<th>Value (sig)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive achievement</td>
<td>Score</td>
<td>Between groups</td>
<td>689.68</td>
<td>2</td>
<td>344.84</td>
<td>71.302</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Within-group</td>
<td>135.417</td>
<td>28</td>
<td>4.836</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant because Sig is less than (0.05)

The values of the (F) test which is found in the cognitive achievement test in football reached (302.71) and at a level of significance of (0.000). It indicates that the significance of the differences in the cognitive achievement test in football between the three learning styles, and to find out the differences between the styles the researchers resorted to using a test the least significant difference (L.S.D) as shown in the following table.
Table 3: The least significant difference test (L.S.D) in the cognitive achievement test for football between the three styles of learning

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Average of difference</th>
<th>(sig) value</th>
<th>Difference significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive achievement in football</td>
<td>Visual mode - auditory mode</td>
<td>6.583</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>visual mode - kinetic style</td>
<td>416.11</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>auditory mode- kinetic style</td>
<td>833.4</td>
<td>0.000</td>
<td>Significant</td>
</tr>
</tbody>
</table>

* Significant because Sig is less than (0.05)

- The significance of the differences between the visual learning style and the auditory learning style in the cognitive achievement test in football and the favor of the visual learning style.

- The significance of the differences between the visual learning style and the kinetic learning style in the cognitive achievement test in football and the favor of the visual learning style.

- The significance of the differences between the auditory learning style and the kinetic learning style in the cognitive achievement test in football and the favor of the auditory learning style.

Discussion

Table (2) showed significant differences between the three learning styles in the cognitive achievement test in football. In favor of the visual learning style, the researchers attribute these differences to the advantages enjoyed by the group of visual students because they see the teacher’s body language and facial expressions, as this leads to a full understanding of the content of the lesson. Also, the owners of this style tend to sit in the front rows to avoid visual obstacles, they learn better than during visual presentations (such as illustrations, maps, data, digital videos, and slides), they also take notes on the skill or concept which leads to the assimilation of information. The visual student’s group learn better through seeing the educational material and the movements of the teacher and how to perform the skill, it is important that they see what the teacher is doing, as they have high skills in receiving, preparing, and processing visual experiences, which makes their awareness of educational experiences better through the media Video. (8)

The various visual aids used by the teacher in lessons such as educational devices make learning easier and interesting and are an effective tool to attract the attention of learners and help them understand different phenomena (9) and the use of visual aids in teaching are one of the ways to improve course progress and give students additional ways to process information and acquire it in an organized way (10) The great scientist Comenius said: The basis of all learning is to reasonably represent the senses so that knowledge can be easily acquired and preserved (11). Visual students can process the largest amount of information more efficiently because the visual representations supported by half of the human brain make students able to integrate ideas, facts, and concepts and save them in an organized way, especially when paired with an educational environment controlled by students, where it enables them to obtain deeper learning and gain different concepts and skills.

As can be seen from Table (3), there are significant differences between the audio students and the kinetic students for the benefit of the audio students, and the researchers attribute this to the fact that the audio students benefit from the valuable information that teachers give in their lectures, in addition to the talk and discussions...
that take place between the students themselves on the one hand, and between the students and the subject teacher on the other hand, where students interpret the meanings behind the teacher’s speech and focus on the tone of his voice and rhythm during the explanation, which reflects positively on their cognitive and skill achievement, and this is unlike the kinetic students who do not find their goal in the curriculum of the subject teacher. Auditory students are described as enjoying speaking and listening, making sounds when reading such as moving lips and whispering, and tending to use phonemes.\(^{(12)}\)

The auditory students listen to the teacher’s explanation and prefer discussion and work in groups. This is common during lessons related to teaching football skills, as the teacher of the subject before each skill allocates a time when he explains that skill in detail while giving time to interact between him and the students such as asking questions and inquiries and answering them. Many students prefer auditory learning style and love to sit in a quiet classroom and listen to the teacher, so they do not use as much of their energy and economize it.\(^{(13)}\)

In the past, the teacher was seen as the core of the educational process, and recent trends seek for looking at the learner and his needs and taking into account his methods of obtaining the information. Therefore the need to understand student learning patterns is increasing in accord with asking for learning within heterogeneous classes.\(^{(1)}\)

\(^{(14)}\)\(^{(15)}\)\(^{(16)}\) see preferred learning styles (VAK) as a side Among the aspects of individual differences that ask for all educational organizations of the need to consider them and deal with students in a way that considers them.

We can consider understanding students’ favorite learning styles as can improve the planning, production, and implementation of educational programs and the development of experiences, and therefore are more beneficial for students’ desires, improve learning, retain information, and speed of retrieval. The effect of students’ learning styles on their achievement, knowledge, or acquisition of concepts with study\(^{(17)}\) all of which dealt with learning styles as independent variables and their impact on acquiring attitudes, skills, and concepts in different subjects.

## Conclusion

The researchers found, through presenting, analyzing, and discussing the results, that there are different educational patterns among the examined students, and that there are significant differences in cognitive achievement in football between students who prefer the visual style and among students who prefer the auditory style and in favor of the visual style. Moreover, there are significant differences in cognitive achievement in football among students who prefer the visual style and among students who prefer the sensory-kinetic and in favor of the visual style, there are also significant differences in cognitive achievement in football between students who prefer the auditory style and among students who prefer the sensory-kinetic and in favor of the auditory style; The researchers recommend that learning Styles should be taken into consideration when developing curricula for physical education, as it is an aspect of individual differences that must be taken into consideration, and teachers should vary in the use of teaching strategies and methods to suit the different learning styles of their students.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

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Guggulsterone Suppresses Ovalbumin- Induced Inflammation in Rat Asthmatic Model

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Abstract

Background: Asthma is an inflammatory airway disease, which is characteristic by wheezing, chest tightness, dyspnea and cough, all symptoms that are occupied with obstruction of respiratory airway. Aim: investigate guggulsterone activity on improve inflammatory events that associated with asthma. Method: 48 healthy rats (albino, male) divided to 6 groups, rats were sensitized with OVA and preparation lung tissue homogenate for measurement of inflammatory parameters by ELISA and RT-PCR. Also, preparation of lung tissue for histopathological examination. Results: All parameters are significant reduction in treated group with guggulsterone than sensitized group. Also, gugglsterone-treated group’s slides with less inflammatory signs compared to sensitized group slide. Conclusion: Guggulsterone improve inflammatory events that associated with asthma.

Key words: gugglsterone, asthma, anti-inflammatory activity , NF-kappaB.

Introduction

Asthma is an inflammatory airway disease, which is characteristic by wheezing, chest tightness, dyspnea and cough, all symptoms that are occupied with obstruction of respiratory airway. The etiology of disease may be many factors. It is believed to be a combination of environmental and genetic factors. On the other hand, other an important inflammatory cytokine is IL-5, that plays a major role in eosinophil differentiation, proliferation, maturation, and migration to tissue sites with survival. Recently, many studies were suggested that IL-33 is present with asthma. After the injury of the lung epithelial cell, IL-33 produces as an alarm signal and stimulates other immune cells to release of IL-5 and 13. In addition, TNF is a majority inflammatory mediator, which is a chemoattractant for eosinophils and neutrophils, and raises the eosinophils cytotoxic activity on endothelial cells. One of most important transcription factor is NF-κB, which plays major roles in the production of pro-inflammatory cytokine.

Guggulsterone (GS) is a bioactive steroid plant, GS is potent ligands to steroid receptor, specifically glucocorticoid receptor. GS has potent anti-inflammatory effects through inhibiting the stimulation of NF-κB (transcription factor) in response to TNF-α and a direct inhibiting the activation of IKK. Also, GS has antioxidant activity, it is inhibited xanthine oxidase and superoxide dismutase. Both enzymes that enhance the production of ROS (reactive oxygen species) that contributed in pathophysiologic with asthma.

Methodology

Chemicals:

Chemicals and drugs used in recent study including ovalbumin (OVA) (Chadwll Heath ESSEX, England), guggulsterone (Xi’an geekee biotech, China), Prednisolone (Pioneer, Iraq), Formaldehyde (37%) (Naturel, Turkey) and AL(OH)₃ (MERK Darmstadt, Germany).
Animals:
Forty healthy rats (albino, male), that weighing (150-300 gm), were taken from animal house of the University of Baghdad/ College of Pharmacy. Rats were put under photoperiods (12:12-hrs dark/light cycle) and controlled temperatures.

Experimental design:

Group I: Rats were administrated distal water orally without sensitization as control group. Group II: Rats were administrated distal water orally with sensitization as positive control group. Group III: Rats were administrated (25 mg/kg) guggulsterone orally with sensitization. Group IV: Rats were administrated (50 mg/kg) guggulsterone orally with sensitization. Group V: Rats were administrated prednisolone (4.12mg/kg) orally with sensitization.

OVA induced-rats by modified protocol of Manal et al (2013), Tong et al (2008), and Michael et al (1999) studies. Rats were sensitization by IP injection of 1mg OVA, 100mg of Al(OH)₃ in 1ml of PBS (phosphate buffer saline) at (1-3) days, then 100mg OVA , 100mg Al(OH)₃ in 1ml of PBS at 6th day, after that animal were challenged at 9th day by glass chamber with the nebulizer with 1% OVA (1gm OVA in 100ml PBS) for 30 minutes daily for 6 days (7), (8) & (9).

Lung tissue preparation:
The left lung was removed. Then, lung tissue was divided for three parts for histopathologic examination, PCR analysis and homogenate samples.

Measurement of gene expression of TNFα and NF-κB by RT-PCR:
Total RNAs were extracted using Trizol (Bioneer, South Korea). Thirty gram of lung tissue was used to extract total RNA for the complementary DNA synthesis using random primers. Reverse transcriptase-PCR was performed following standard procedures. The primer pairs of the expected products were as follows (forward and reverse, respectively): TNF-alpha, 5′- CTTCTCATTCTGCTGTTG-3′ and 5′- TGATCTGAGTGAGGCTG-3′, NF-κB 5′- CTACGAGACCTTCAAGAGCATC -3′ and 5′- GATGTTGAAAAAGGCATAGGCC -3′, and GAPDH, 5′- TCCAGTATGACTCTACCAGG -3′ and 5′- CACGACATACTCACGACCAG -3′. Amplification products were resolved by 1.0% agarose gel electrophoresis, stained with ethidium bromide and photographed under ultraviolet light. Primers were purchased from Bioneer, South Korea. Real-Time PCR was performed using AccuPower GreenStar qPCR PreMix according to the manufacturer’s instructions (Bioneer, Cat No: K-6210).

Histopathological examination of lung tissue:
The left lung is removed from all the experimental rats. Then it was washed with normal saline solution for preparation tissue to histopathological examination. Then, the washed lung tissues were fixed with formaldehyde (10% of formaldehyde in water). The sample was dried from water by Xylene. This process is done overnight and automated. Finally, molten wax was surrounded the specimen in the container and solidification by cooling and embedding in the wax block. Then, the thin section slide was stained and mounted by the protective cover slip.

Statistical Analysis
Statistical Package for the Social Sciences (Spss, version 25) statistic program was used in the present study for data analyzing. An unpaired Student t-test and one-way ANOVA test used for comparison between groups for finding significant statistic different.

Results
Effect of guggulsterone on IL-4, IL-5 and IL-33 levels in lung tissue homogenate:
IL-4, IL-5 & IL-33 are inflammatory cytokines. IL-4, IL-5 & IL-33 levels (mean ± Std. Error) for rats of group II (positive control) (78.3 ± 5.2, 97 ± 19.7 & 82.4 ± 4.7, respectively) were significantly elevation (p<0.001) than group I (control group) (19.5 ± 5.1, 26.8 ± 1.6 & 35.9 ± 4, respectively) as showed in figure 1. The other significant different in IL-4, IL-5 & IL-33 levels for rats of treated groups III (25 mg/kg guggulsterone) (19.2 ± 2.8, 43.5 ± 11 & 41.4 ± 3.9), IV (50 mg/kg guggulsterone) (16.6 ± 2.4, 32 ± 13.4 & 37.8 ± 3.1) & V (4.12 mg/kg predinsoline) (27.6 ± 2.4, 50.7 ± 6.2 & 46.8 ± 5.4, respectively) were significant reduction (p<0.001) as compared to senitized group II (positive control group) (78.3 ± 5.2, 97 ± 19.7). Also, IL-4 levels for rats of group IV (16.6 ± 2.4) than other treated group V (27.6 ± 2.4).

Values are indicated as means ± Std. Error (n=8) for each group. Group I: Control group, Group II: Positive control group (with sensitization), Group III: Guggulsterone (25 mg/kg) with sensitization, Group IV: Guggulsterone (50 mg/kg) with sensitization, Group V: Predinsoline (4.12 mg/kg) with sensitization. *** symbol referred to significant different (p<0.001) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05).

Effect of guggulsterone on TNFα levels in lung tissue homogenate:

TNF levels (means ± Std. Error) in tissue homogenate for rats of sensitized group II (positive control group) (210.9 ± 26) was significantly elevated (p<0.01) than group I (control group) (94.1 ± 21.1) as showed in figure 2. Furthermore, TNF levels in tissue homogenate for rats of treated groups (III, IV & V) (124.2 ± 23.3, 113.1 ± 20.1 & 115.4 ± 10.4) were significantly reduction (p<0.05) than IL-33 levels in tissue homogenate for rats of sensitized group II (positive control group) (210.9 ± 26).
Figure 2: Inhibitory effect of guggulsterone on OVA-induced TNF-alpha expression in lung tissue homogenate.

Values are indicated as means ± Std. Error (n=8) for each group. ** symbol referred to significant different (p<0.01) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05).

Effect of guggulsterone on IgE levels in lung tissue homogenate:

IgE is immune marker appeared with an asthmatic pathway. IgE levels in lung tissue homogenate (means ± Std. Error) for rats of group II (sensitized, positive control group) (176.7 ± 7.5) was significantly increased (p<0.001) than group I (control group) (52.8 ± 5.7) as noted in figure 3. Besides, there were significant reduction (p<0.001) in IgE levels in tissue homogenate (means ± Std. Error) for rats of treated groups (III, IV & V) (44.9 ± 8.1, 58.7 ± 2.1, & 47.6 ± 7.3, respectively) compared to sensitized group II (positive control group) (176.7 ± 7.5).

Figure 3: Inhibitory effect of guggulsterone on OVA-induced IgE in lung tissue homogenate.
Values are indicated as means ± Std. Error (n=8) for each group.*** symbol referred to significant different (p<0.001) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05).

**Effect of guggulsterone on gene expression of TNFα and NF-κB:**

Gene expression of TNFα and NF-κB (means ± Std. Error) for rats of group II (positive control group) (1.94 ± 0.21, 3.33 ± 0.43) were significantly raised (p<0.001) than group I (control group) (0.41 ± 0.22, 0.7 ± 0.16) as appeared in figure 4. Also, gene expression of TNFα for treated rats of group V was significantly elevation (p<0.05) in compared to group I (control group). There were significant reduction (p<0.001) between gene expression of TNFα and NF-κB (means ± Std. Error) for rats of treated groups (III (0.52 ± 0.23, 0.94 ± 0.08), IV (0.74 ± 0.17, 0.98 ± 0.11) & group V (1.07 ± 0.21, 1.11 ± 0.16) than sensitized group II (positive control group) (1.94 ± 0.21).

**Figure 4: Effect of gugglsterone on gene expression of TNFα & NF-Kβ in lung tissue.**

Values are indicated as means ± Std. Error (n=8) for each group. *** & * symbols referred to significant different (p<<0.05) compared to control group, unpaired test student t-test. Groups with non-identical letters are significantly different (p<0.05).

**Histological assessment of rats’ lung tissue for all studied groups:**

**Figure 5** showed that (D) the cross section of lung tissue for rats group I (control group) and noted that alveolar sac was cleared. Inflammatory cells were not agglomerate in the interstitial tissue. While, (E) group II (positive control group) manifested inflammatory cells were agglomerated in interstitial tissue. Also, Alveolar sac was filled with inflammatory cells. On other hand, (F), (G) & (H) treated groups (III (25 mg/kg guggulsterone), IV (50 mg/kg guggulsterone) & V (4.12 mg/kg predinsoline)) appeared that very less number of inflammatory cells agglomerated in interstitial tissue and alveolar sac was cleared.
Discussion

Asthma is one of the inflammatory and chronic airways disorders, which is manifested that an adaptive and innate immune systems with each other with epithelial cells to produce BHR, overproduction of mucus and airway wall narrowing and remodeling. Predinsoline is traditional ant-inflammatory drug, which is used in this study for comparison pharmacological activity with gugglsterone.

In the current study, IL-4 and IL-5 are proinflammatory mediators, which are higher for rats of sensitized group than control group. Bagnasco et al (2016) study revealed that IL-4 & IL-5 levels in lung tissue are significantly higher in asthmatic group compared to control group. On other hand, IL-4 & IL-5 are significantly reduction with treated groups with guggulsterone due to guggulsterone has anti-inflammatory activity due to its steroid structure and its ability to bind with glucocorticoid receptor in target nucleus and inhibit the pro-inflammatory transcription factors, AP-1 and NFkB and reduced the gene expression of IL-4& IL-5. The other inflammatory mediators is IL-33and it’s level in tissue homogenate for rats of sensitized group is significantly more than control group I. This results in line with the other previous study, Allinne et al (2019). IL-33 levels reduce in treated groups because guggulsterone is occupied with nuclear GR in target cells that lead to increase of DUSP1 expression lead to induce of feedback inhibition of MAPKs and reduce of IL-33 inflammatory signaling pathway.

TNF-α is a powerful proinflammatory cytokine that mediated to inflammatory response of asthma. Kumar et al (2017) study appeared that TNF is higher with asthmatic group in compared to normal group. Similarly, Al-Quraishi (2013), & Froidure et al (2016) revealed that higher level of IgE in asthmatic group than control group. While, Treated groups with guggulsterone that showed significant reduction in TNFα & IgE levels due to guggulsterone has anti-inflammatory activity mediated by glucocorticoid receptor.

Furthermore, TNFα and NF-kB could be measured gene expression in mRNA through RT-PCR technician. In this study, Gene expression of TNFα and NF-kB
are significantly elevation with sensitized group than control group. Busse et al (2005) & Ather et al (2011) studies appeared that quantitative expression of TNFα and NF-κB are higher in asthmatic group in compared to normal subjects. On other hand, guggulsterone has anti-inflammatory activity lead to suppress the gene expression of proinflammatory cytokines including IL-1β, IL-6, IL-8, and TNF-α. Consequently.

Histopathologic results in the recent study appeared inflammatory cells were agglomerated in interstitial tissue of asthmatic lung tissue for rats of sensitized group. Furthermore, Alveolar sac was filled with inflammatory cells and the wall thickness of bronchiole was increased due to pathogenesis pathway of asthma. Guggulsterone with anti-inflammatory activity appeared that lung tissue returned to the normal state with very less number of inflammatory cells agglomerated in interstitial tissue.

Conclusion

Guggulsterone is natural material that has many anti-inflammatory activities through several mechanisms that mediated by steroid receptors and affected on many inflammatory mediators. In future, further many studies that performed guggulsterone could be used for treatment of airway and other inflammatory diseases.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Study the Role of Helicobacter Pylori Infection in a Group of Iraqi Patients with Colorectal Cancer

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Abstract

Background: Increased developed of colorectal tumor may be linked with Helicobacter pylori. However, the underlying mechanisms were still uncertain.

Objective: Helicobacter pylori infection in the world, in particular in developing countries, is one of most common chronic bacterial infections, This bacterium is responsible for many diseases such as gastritis, peptic ulcer, lymphoma and in some patients may lead to gastric cancer, it is in the world has known 2nd major cause of death from cancer. H.pylori infection may likely raise the risk of colorectal cancer, according to recently published studies. The aimed of this study to detected the role of H. pylori in colorectal cancer.

Methods: Serum of 50 colorectal cancer and colorectal normal with positive H. pylori infection patients were estimated by 14Urea breath test and 25 subjects with no any inflammatory disease as the control group. from each patient were collected Blood samples to investigated of H. pylori specific immunoglobulin G (IgG) and CagA IgG antibodies by using enzyme-linked immunosorbent assay (ELISA).

Results: A total of 50 patients in this study 25 had colorectal cancer and 25 had colorectal normal with H. pylori infection (immunoglobulin G (IgG) P ˂ 0.01 and CagA IgG antibodies P < 0.01) were significantly more prevalent in the patients with colorectal cancer and colorectal normal with Helicobacter pylori infection compared with the healthy controls (P= 0.00019, P= 0.00022 respectively) The relation was statistically non-significant between the antibody seropositivity and gender but Highly significant with age.

Conclusion: The findings indicate that Helicobacter pylori infection can be regarded as a contributing influence for progressive tumor in colorectal.

Key Words: colorectal cancer, Helicobacter pylori-Infection, Serum, Immunoglobulins

Introduction

Colorectal cancer (CRC): One of the world’s most common cancers and the third-largest cause of death from cancer. As shown in 2018 accordingly to epidemiological survey, 1,800,977 new cases with colorectal cancer and 861,663 mortality related to colorectal cancer were registered (1). The International Cancer Research Agency has listed H.pylori as Group I carcinogenic in gastric cancer (2). It’s generally exists in the stomach and that is microaerophilic gram negative (-ve) bacterium (3). Warren and Marshall first cultivated it in 1981 (4). than half of the population are infected with this pathogens and It was most common infection in the world (5). H.pylori transmitted by contaminated water and food It acquired mainly in early childhood and may remain untreated for life, but it also can be acquired in adults (6). Infection prevalence varies in worldwide depending on socioeconomic factors and hygiene rates (7). Most people will not have any problems with the infection but in some H.pylori long-lasting inflammation in the stomach may cause with peptic ulcer disease and mucosa associated lymphoma (8). A major risk for stomach adenocarcinoma occurrences is Helicobacter pylori (9).

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The cytoxin-associated gene (CagA) may also contribute to cause inflammation and carcinogenic effect (10). The region of CagPAI approximately 40-kb of H. pylori chromosome. The disease of H. pylori can be enhanced by cagA pathogenicity genes in West countries, around 50–70% of H.pylori strains carry it (11). Persons with H.pylori CagA-positive strains infection produce CagA protein circulating antibodies, which are used as diagnostic markers (12). In extra gastric diseases has been also proposed that play an important role, the colorectal was the major extra-gastric organ with H.pylori that expressed of CagA tumor formation and increase developing of colorectal cancer (13).

Colorectal tissue infection with H. pylori may doesn’t contribute in order to elevated the threat of colorectal cancer directly (14). One hypothesis is that gastric Cytotoxin-linked gene A Protein in Helicobacter pylori infection triggers a rise in serum gastrin levels by overproduction of IL8 that high level of gastrin may lead to hypergastrinemia; (15). That is expected to be Intestinal mucosal proliferative consequence carcinogenesis involves cell inflammation and deregulation cycle (16). H.pylori carcinogenesis involves inflammation and the deregulation of the cell cycle by means of the Cytotoxin-associated gene A (CagA) H.pylori protein, binding SHP2 (a human), and acting on it the oncoprotein-based phosphatase which can produce tumor cell growth (17). Links between H.pylori and colorectal tumor either indirect such as correlating increased CagA+ levels and these lesions (18) or increased gastrin levels (19).

The aim of present study to examine the correlation between H. pylori and progressive tumor in colorectal.

### Material and Method

The present study included 50 patients which divided in to (25 were colorectal cancer, 25 were colorectal normal with H. pylori infection) and 25 subjects without any pathological findings as a healthy control group. The current case-control study was attending the gastroenterology & hepatology teaching Hospital/medical city, the endoscopy unit of Ramadi teaching Hospital, and endoscopy unit of Fallujah general Hospital unit from a period September 2019 to February 2020. Arabian patients of Iraq in the Middle East. Patients were chosen with the assistance of surgeons in hospitals. Information has been registered for every patient. Used H2 antagonist or proton-pump inhibitors treatment, former stomach operation and malignancy at another site was exclusion from current study criteria. There are various H. pylori diagnostic tests including invasive tests (endoscopy, biopsy, histopathology, rapid urease, and PCR) and noninvasive tests (respiratory urease, ELISA and stool antigen). Due to their high sensitivity, specificity and simplicity, the current research used the ELISA system. To detected the presence of anti-Helicobacter pylori antibodies. Five ml blood was obtained, from 75 for each group subjects their sera has been tested for Anti-Helicobacter pylori IgG, IgG (CagA) by using enzyme-linked immunosorbent assay (ELISA). Chi-square analyses were conducted for statistical analysis. P values below 0.05 were statistically significant and greater than 0.05 were non-significant.

### Results and Discussion

The distribution of patients according to gender was higher in the male patients with colorectal cancer and colorectal normal with Helicobacter pylori infection than female but no significant differences (P= 0.64), In Table (1)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Colorectal cancer</th>
<th>Colorectal normal and H. pylori +ve</th>
<th>Healthy control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>32.0</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>68.0</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

C2 = 0.878 P= 0.64 P > 0.05 Non-significant
Our study agreement with Teimoorian F, et al. (20) which conducted The rate of colorectal cancer was (64%) in males and (35%) in females. whereas Fujimori S, et al (21) colorectal adenocarcinoma incidence was higher incidence in women than men. High risk for development of colorectal cancer with \textit{H. pylori} infection may due to Adult females typically have better innate and adaptive immune responses than males.

In Table (2) The study that has been conducted the highest incidence of colorectal cancer infection was shown (56%) who belonged to the age group (59-69) years, while the other patients were within the age groups (48-58) and (37-47) years in rate (28%) and (12%) respectively. The highest rate of infection in colorectal normal patients were found in (36%) with age group (48-58) year followed by those within the age group (59-69) and (37-47) years in rate (28%) and (16%) respectively. the results highly significant \( P. value = 0.0005 \).

Table (2) distribution of patients according to age

<table>
<thead>
<tr>
<th>Age groups (Years)</th>
<th>Colorectal cancer</th>
<th>Colorectal normal and \textit{H. pylori} +ve</th>
<th>Colorectal normal and \textit{H. pylori} -ve</th>
<th>Total</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25</td>
<td>0(0%)</td>
<td>2(8%)</td>
<td>10(40%)</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>26-36</td>
<td>1(4%)</td>
<td>3(12%)</td>
<td>7(28%)</td>
<td>11</td>
<td>14.67</td>
<td></td>
</tr>
<tr>
<td>37-47</td>
<td>3(12%)</td>
<td>4(16%)</td>
<td>4(16%)</td>
<td>11</td>
<td>14.67</td>
<td></td>
</tr>
<tr>
<td>48-58</td>
<td>7(28%)</td>
<td>9(36%)</td>
<td>2(8%)</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>59-69</td>
<td>14(56%)</td>
<td>7(28%)</td>
<td>2(8%)</td>
<td>23</td>
<td>30.66</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>75</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

\( C^2 = 33.084 \ P. value = 0.0005 \) Highly Significant (HS)

Colorectal cancer that combine with \textit{H. pylori} infection in our study appeared significantly higher in the old age people that agrees with previous studies like Zhao Y, et al (22). In colorectal normal with \textit{H. pylori} positive patients this study in agreement with Al-Jubori et al. (23), which demonstrated the same range of \textit{H. pylori} infection at age between (48-58) in Duhok province while not match with study(24), who recorded the highest infected group ranged between (20 - 39) years old. Highest rate of infection in old age may be due to low immunity state. In Table (3). The anti-helicobacter pylori IgG antibodies was highly significantly higher in patients with colorectal cancer and colorectal normal with \textit{Helicobacter pylori} infection compared with the healthy controls (\( P = 0.00019 \)).
Table (3) Detection of Anti-Helicobacter pylori-IgG in patients and control.

<table>
<thead>
<tr>
<th>Seroprevalence of IgG Antibodies</th>
<th>Colorectal cancer</th>
<th>Colorectal normal and H. pylori +ve</th>
<th>Colorectal normal and H. pylori - ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>IgG +ve</td>
<td>16</td>
<td>64.0</td>
<td>19</td>
<td>76.0</td>
</tr>
<tr>
<td>IgG -ve</td>
<td>9</td>
<td>36.0</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

C2 = 26.351  P= 0.00019  P < 0.01 Highly Significant

Our study in agreement with study by (Fireman et al., 2000), that’s reported the highest rate of anti-helicobacter pylori IgG in the colorectal cancer than in healthy control, but different with Buso et al. (25), which reported H. pylori IgG antibodies was (71%) in the colorectal cancer group and patients in the control group (65%), the difference having non-statistical significance.

H. pylori have been found in the whole world repeatedly most people diagnosed with H. pylori are asymptomatic, the infection acquired for lifespan(26). It is rarely spontaneously eradicated with any therapy. According to Table (4) CagA IgG antibodies was significantly higher in the patients with colorectal cancer and colorectal normal with Helicobacter pylori infection compared with the healthy controls (P= 0.00022).

Table (4) Detection the levels of (CagA) IgG antibodies among patient groups and control group.

<table>
<thead>
<tr>
<th>CagA-IgG Antibodies</th>
<th>Colon cancer and</th>
<th>Colon normal and H. pylori +ve</th>
<th>Colon normal and H. pylori - ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>68.0</td>
<td>8</td>
<td>32.0</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>32.0</td>
<td>17</td>
<td>68.0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

C2 =26.04  P= 0.00022  P < 0.01 highly Significant

Current study agrees with Shmuely et al, (27), which showed that in patients with colorectal cancer, seropositive CagA rate was about twice higher than in control and statistically significant, but different study with Zumkeller
et al\(^{(28)}\) who has been investigated no link between \textit{Helicobacter pylori} CagA seropositivity and incident colorectal adenocarcinoma.

There is still little understanding of the mechanism by which \textit{H. pylori} elevated the risk of colorectal cancer. CagA has a pathogenic function in increased risk of gastric cancer \(^{(29)}\). CagA toxin induces serum gastrin secretion, which can serve as a growth hormone in colonic cells \(^{(30)}\) and can cause hypergastrinemia, suggesting an appropriate mechanism for the carcinogenicity of that organism. Moreover, infected with CagA-positive \textit{Helicobacter pylori} strains are correlated with the increased risk of atrophic gastritis developing \(^{(31)}\). Secondary a tropical gastritis to \textit{Helicobacter pylori} infection is associated with decreased acid production which makes the intestinal tract more common to a large number and variety of microbial species, which are associated with tumor in colonic growth \(^{(32)},^{(33)}\). A number of observational studies have investigated the correlation between \textit{H.pylori} serum positive and colorectal cancer risk \(^{(21)},^{(34)}\) and other found no relationships at all between this infection and the cancer \(^{(35)}\).

Another study by PCR The incidence of \textit{Helicobacter pylori} in colorectal adenocarcinoma tissue was significantly higher in contrast with the normal colorectal tissue \(^{(36)}\) in particular CagA positive strains the gastric colonized mucus trigger D gastric antrum cell deficiencies. Lead to elevated of gastrin that act as mitogen proliferative of tumor in colorectal tissue\(^{(37)}\).

Conclusion

The research indicated that a significant link between CagA-positive \textit{Helicobacter pylori} and colorectal cancer; development by different mechanism. Nevertheless, Much farther studies with larger samples are required to accurately evaluate the role of \textit{H. pylori} in these pathologies. Shed further bright on their probable role in colorectal carcinogenesis with \textit{Helicobacter pylori} infection by study other virulence factor VacA cytotoxin and BabA adhesion.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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A Reliable Quantification Method for Trimethoprim in Pharmaceutical Samples by HILIC-HPLC

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Abstract

Trimethoprim is sometimes used in conjunction with sulfonamides for drug therapies. A practicable method has been developed to determine trimethoprim in pharmaceutical formulations by hydrophilic interaction liquid chromatography coupled with an ultraviolet detector. The development and investigation of a new HLIC assay of trimethoprim. The separation was made in the HALO-HILIC column using a NaOAc/HAc buffer (40 mM-pH 4.75) with acetonitrile (10:90) (v/v) as mobile phase and was quantified by UV detection at 280 nm. The limit of quantification was about 0.0242 ppm and the limit of detection 0.008 ppm. The calibration curves were linear in the investigated range (0.01-3 ppm). The recovery of trimethoprim was 99-102.5%. The method was applied in pharmaceutical formulations.

Keywords: Trimethoprim, HILIC, Tablet, syrup, UV-detection; toxicity

Introduction

Trimethoprim is an antibiotic used exclusively in bladder therapy. For the middle ear and traveler’s diarrhoea, there are other uses. In people with HIV/AIDS, pneumocyst pneumonia can be treated with sulfamethoxazole or dapsone (1, 2). Trimethoprim class of compounds is diaminopyrimidine. It has an inhibitor of sulfonamide dihydrofolate reductase, commonly prescribed synergistic antimicrobial agents mainly used in the prevention and treatment of urinary tract infections (3). And used to treatment Pneumocystis carinii pneumonia, otitis media, shigellosis, salmonellosis, and chronic bronchitis (4). Trimethoprim was available from 1969 Sulphametboxazol combined (Co-trimoxazole) but has been marketed in the US, Scandinavia since 1980 (5, 6).

For the determination of trimethoprim in pharmaceutical or biological samples, several HPLC procedures were identified (4, 7-16). Hydrophilic interaction liquid chromatography (HILIC) was a variant of normal stage liquid chromatography, but the separation mechanism employed in HILIC was more complex than that in NP-LC. Alpert proposed the abbreviation HILIC for the first time in 1990 (17). Since 2003, the number of HILIC publications has significantly increased (18). HILIC, like NP-LC, employs traditional stationary polar phases like silica, amino, or cyano (19-23). However, the mobile phase used in the mode RP-LC is identical (18, 22, 23). HILIC technology has thus recently begun to growing dramatically in the determination of pharmaceuticals, nucleosides, carboxylic acids, inorganic ions, dansyl-amino acids, and flavonoids by Rasheed and its co-workers (24-38). The goal of this study was therefore to develop and validate a simple, rapid, and sensitive HILIC method in pharmaceutical samples to determine trimethoprim.

Materials and Methods

Chemicals and reagents:

In purifying solutions, Millipore filters (0.22 μm) were used. From Sigma-Aldrich obtained trimethoprim, acetonitrile (ACN) and sodium acetate as far as the chemicals are concerned. 0.1 μs/cm (System-US Millipore) of Millipore water conductivity was used. Tablets and syrups of six different commercial companies, as follows: tablets (Supreme 500 mg-Ajanta-India, Septrin 500 mg-Aspen-Germany, Methyprin 500 mg-SDI-Iraq), syrups (Septrin Paediatric Suspension
100 ml-Aspen-Germany, Bactrim 100 ml-ASIA Pharmaceutical Industries-Syrian, Bactrim 100 ml-Pioneer-Iraq).

**Instrumentation and chromatography**

Chromatography was performed on the Merck-Hitachi HPLC System consisting of an L-6200 gradient pump; the Rheodyne valve allows injection 20 μL and UV-visible L-4200. A HILIC HALO® 2.7 column (100 mm-2.1 mm) was used. The data were collected using N2000 workstation software to empower chromatography manager. The mobile phase composed of acetonitrile with acetate buffer (40 mM-pH 4.75) (90:10) (v/v). The mobile phase was filtered through 0.2 μm Nylon filter membranes. The analysis was carried out under gradient conditions using a flow rate of 0.5 ml/min at room temperature. The chromatogram of separation and determination trimethoprim was detection at 280 nm.

**Results and Discussion**

**Optimizing the separation of trimethoprim:**

As a pharmaceutical model, trimethoprim has been selected to test the HILIC retention mechanism using HALO 2.7 column by applying the acetate buffer with ACN content as eluent. At 90% ACN and 40 mM-pH 4.75 of acetate buffer, the chromatogram was obtained (Figure 1). The systemic variability of the contents of the ACN is increasing in mobile phase compounds between 50% and 95%; the concentration of eluent between 10 mM and 80 mM with a pH between 3 and 5.5.

![Figure 1: Chromatogram for the separations of trimethoprim using Halo column](image)

Separation of trimethoprim with varying ACN content

Eluent ACN effect on trimethoprim retention was observed at 40 mM-pH 4.75 acetate buffer. Trimethoprim hydrophilic interaction behavior appears to increase by 50% to 95% in the eluent ACN ratio, with the trimethoprim retention factor increasing. Trimethoprim hydrophilicity is the explanation for this behavior; trimethoprim’s HILIC behavior is shown (Figure 2a), which was attributed to the trimethoprim log P_{OW} (-0.16) (39).

Separation of trimethoprim with varying buffer concentration
In 10-80 mM (pH 4.75) of eluent, the effect of the acetate buffer is recorded at an eluent ACN of 90 percent. The findings appear in (Figure 2b). The trimethoprim retention factor in the column should increase the rising buffer concentration in the acetate eluent. The hydrophilicity of trimethoprim is the explanation for trimethoprim behavior. The stationary process of the HILIC material is closely related.

Separation of trimethoprim with varying eluent pH

A shift in eluent pH can be used to boost the next composition of the mobile phase. The eluent pH must be modified to completely distinguish the trimethoprim in HILIC mode. At a constant buffer of 40 mM and ACN of 90%, the pH was improved from 3 to 5.5. The retention time decreases as shown in Figure 2c. This is because the amino group in trimethoprim is deprotonated.

**Figure 2:** Study separation mechanism of trimethoprim (a) Effect of ACN content (b) Effect of buffer concentration (c) Effect of pH (d) The calibration curve.

**Calibration graph:**

The calibration trimethoprim curve is developed by plotting the trimethoprim concentration against the peak area and showing the concentration range (0.01-3 ppm) as Figure 2d shows.

Statistical data information:

A detailed evaluation under HILIC conditions and a monitor of statistics in Table 1 were used for the corresponding calibration curve of trimethoprim. Precision and accuracy were evaluated on the same day and different days with RSD% and Rec.% were evaluated. The relatively small defaults and high recuperation values indicate that the proposed method is successful (Table 2).
Table 1: The results for standard trimethoprim curve are checked using the HALO column.

<table>
<thead>
<tr>
<th>Function</th>
<th>HALO method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearitya (ppm)</td>
<td>0.01-3</td>
</tr>
<tr>
<td>Regressiona equation</td>
<td>( y = 303.06 + 1011.40^* x )</td>
</tr>
<tr>
<td>R2</td>
<td>0.9999</td>
</tr>
<tr>
<td>LODi(ppm)</td>
<td>0.008</td>
</tr>
<tr>
<td>LOQf(ppm)</td>
<td>0.0242</td>
</tr>
</tbody>
</table>

Table 2: Trimethoprim statistical results on the same day as on various days.

<table>
<thead>
<tr>
<th>Same-Day Analysis n=4</th>
<th>Day-to-Day Analysis n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimeth. Takenf (ppm)</td>
<td>Trimeth. Foundf (ppm)</td>
</tr>
<tr>
<td>Trimeth. Foundf (ppm)</td>
<td>% Rec.</td>
</tr>
<tr>
<td>0.5</td>
<td>0.510</td>
</tr>
<tr>
<td>1.5</td>
<td>1.492</td>
</tr>
<tr>
<td>2.0</td>
<td>1.980</td>
</tr>
</tbody>
</table>

Determination of trimethoprim in drug samples:

In evaluating trimethoprim in six of the pharmaceutical forms, the approach developed is used successfully, with the results described in Table 3.

Table 3: Pharmaceutical appliance of the proposed method of trimethoprim determination.

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Company</th>
<th>Present (mg)</th>
<th>Get it (mg)</th>
<th>%Rec.</th>
<th>%RSD n=4</th>
<th>% Erel.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septrin Paediatric Suspension 100 ml-</td>
<td>Aspen-Germany</td>
<td>40</td>
<td>39.87</td>
<td>99.67</td>
<td>0.23</td>
<td>-0.33</td>
</tr>
<tr>
<td>Syrup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bactrim 100 ml- Syrup</td>
<td>ASIA Pharmaceutical</td>
<td>40</td>
<td>40.10</td>
<td>100.25</td>
<td>0.42</td>
<td>0.25</td>
</tr>
<tr>
<td>Industries-Syrian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bactrim 100 ml- Syrup</td>
<td>Pioneer-Iraq</td>
<td>40</td>
<td>39.77</td>
<td>99.42</td>
<td>0.66</td>
<td>-0.58</td>
</tr>
<tr>
<td>Supreme 500 mg-Tablet</td>
<td>Ajanta-India</td>
<td>80</td>
<td>80.23</td>
<td>100.23</td>
<td>0.55</td>
<td>0.23</td>
</tr>
<tr>
<td>Septrin 500 mg-Tablet</td>
<td>Aspen-Germany</td>
<td>80</td>
<td>79.80</td>
<td>99.75</td>
<td>0.44</td>
<td>-0.25</td>
</tr>
<tr>
<td>Methprim500 mg-Tablets</td>
<td>SDI- Iraq</td>
<td>80</td>
<td>79.60</td>
<td>99.37</td>
<td>0.76</td>
<td>-0.63</td>
</tr>
</tbody>
</table>
Such findings have been compared to the results produced about those obtained in the United States Pharmacopeia protocol \(^{(40)}\) to determine the competence and efficiency of the HILIC approach. The results of the t-test and F-test variance-ratio (Table 4) which were confidential to 95% were used for data analysis. The measured values of t and F did not surpass the theoretical values so that the accuracy of the determination of trimethoprim in six pharmaceutical types does not substantially vary in both methods.

**Table 4: The comparison of the proposed method with the standard method for trimethoprim analysis by investigating t- and F-statistical tests.**

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Halo method</th>
<th>Standard Method (40)</th>
<th>t-Test (theor.)</th>
<th>F-Test (theor.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septrin Paediatric Suspension 100 ml-Syrup</td>
<td>99.67</td>
<td>99.88</td>
<td>0.5722 (2.2281)</td>
<td>0.4058 (5.0503)</td>
</tr>
<tr>
<td>Bactrim 100 ml- Syrup</td>
<td>100.25</td>
<td>100.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bactrim 100 ml- Syrup</td>
<td>99.42</td>
<td>99.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supreme 500 mg-Tablet</td>
<td>100.23</td>
<td>100.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septrin 500 mg-Tablet</td>
<td>99.75</td>
<td>99.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metheprim500 mg-Tablets</td>
<td>99.37</td>
<td>99.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

A new validated HILIC method was developed to determine trimethoprim in syrups and tablets. The HILIC method has demonstrated compliance with the ICH Harmonized Tripartite Guideline. To evaluate low ppm trimethoprim ranges, a HILIC method was developed. The suggested method was simple, fast, and sensitive enough. HILIC interaction with trimethoprim is seen in the stationary HALO phase. It is due to the Octanol-water partition coefficient value of trimethoprim.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

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Assessment of Serum Concentration of Ghrelin and Obestatin in *Giardia lamblia* Infected Patients: A Case Control-Study

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Abstract

Background: Globally, *Giardia lamblia* is one of the major causes of diarrhea in humans and it is the commonest reported gastrointestinal parasite. Thus, the present research work aims to investigate the serum concentration of ghrelin and obestatin in patients with giardiasis. **Objective:** The current study was designed to determine of *G. lamblia* infection effects on some biomarkers such as ghrelin and obestatin. **Methods:** The study was conducted on 48 patients infected with *G. lamblia* and 48 healthy persons, who attended the general hospital of AL-Hakeem in Najaf city from September to December 2018. **The results:** The results showed a highly significant decrease (P<0.0001) in serum concentration of ghrelin and a highly significant increase (P<0.0001) in serum concentration of obestatin in *G. lamblia* infected patients (28.85ng/ml ±3.2) and (186.53ng/ml ± 1.56) respectively, in comparison to the control group (81.39ng/ml ± 3.8) and (92.89ng/ml ±2.9) respectively, with a significant positive association between the concentrations of ghrelin and obestatin (r = 0.0954). **Conclusion:** Ghrelin and obestatin are important physiological biomarkers that can support the diagnosis of *Giardia lamblia* parasite.

Keywords: *Giardia lamblia*, giardiasis, ghrelin, obestatin, Diarrhea, Patients.

Introduction

Giardiasis is a gastrointestinal infection caused by a zoonotic flagellated protozoan parasite *Giardia lamblia*. This parasite replicates in a luminal non-invasive extracellular way into the small intestine of humans and other vertebrates, that may cause a diarrheal disease [¹]. Worldwide, *G. lamblia* is the third major infectious cause of diarrheal disease with more than 300 million detected cases annually [²].

Giardiasis in humans may be asymptomatic or associated with diarrhea, flatulence, abdominal cramp, malabsorption, bloating, and weight loss. Severe infection leads to malabsorptive diarrhea with bulky and greasy stools [³].

Because the gastrointestinal tract parasitic infection produces injurious and harmful effects on the tissues and the physiology of the host, various mechanisms that lead partially or completely to the loss of appetite, the loss weight, the malabsorptive diarrhea or afebrile status should be assessed [⁴].

Although many studies regarding intestinal protozoa as *G. lamblia* concentrate on the anorexia; to date, an appetite peptide level, ghrelin, and its derivative obestatin in the parasitic infections of the GIT have not been investigated completely.

Ghrelin is a 28-amino acid peptide, and is produced and released mainly by the stomach. Ghrelin is a main appetite regulator through initiation of meal after sensing of nutrient [⁵]. In addition, ghrelin control homeostasis of glucose by inhibition the secretion of insulin and control the hepatic output of glucose. Ghrelin signaling controls homeostasis of energy by reducing thermogenesis to decrease expenditure of energy. [⁶].

Obestatin is a 23-amino acid peptide hormone released from the stomach. Apposite to ghrelin, that causes an increase in appetite and weight (obesity), obestatin appears to act as an appetite decreasing hormone (anorectic hormone), reducing intake of food and decreasing weight gain. Obestatin also participated in ameliorating memory, controlling sleep, affecting the proliferation of cell, elevating the pancreatic juice enzyme secretion and preventing glucose-induced
insulin secretion.

Regarding the orexigenic function (appetite stimulant) of ghrelin and the anorectic effect (appetite reducer) of obestatin [7], this study hypothesized there was a reduction in the level of serum ghrelin and an increase in the serum level of obestatin in patients with giardiasis. Ghrelin is termed the ‘hunger hormone’ because it stimulates appetite, while Obestatin is an anorectic peptide.

In addition, the above functions of ghrelin and obestatin may be affected by the change in the serum levels of these two hormones which may explain many aspects in the pathophysiology of some of the giardiasis clinical features as loss of appetite, loss of weight, absence of fever, failure to thrive in children and other signs and symptoms. Therefore, the aim of the present study was to evaluate the serum concentration of ghrelin and obestatin.

The results of the current study may supply an important information regarding the effects of ghrelin and obestatin in giardiasis.

**Materials and Methods**

During the period from September to December 2018, a case-control study was designed for 48 patients (28 males and 20 females) with giardiasis and 48 healthy persons, who had no clinical evidence of any type of diseases (26 males and 22 females) as a control group.

The patients were randomly selected from patients who attended AL-Hakeem hospital, Najaf Province.

Patients have included if the general stool examination was positive for *Giardia lamblia* parasite, from both genders male or female of any age.

Any patient with giardiasis on treatment, the patient with chronic loss of appetite and/or weight for any cause rather than giardiasis, febrile patient, a diabetic patient, obese patient, and/or patient with mixed intestinal parasitic infection were excluded.

The Kufa Medical College Ethical Committee approved the protocol of this study.

**Stool Samples Collection:** Each stool sample was collected in a clean screw cap stool containers, labeled with the number and date of collection.

**Detection of *Giardia lamblia* Parasite:** Microscopic diagnosis of *Giardia lamblia* was performed immediately after the collection of the stool sample. A small amount of stool specimen was processed and for each stool specimen, normal saline and Lugol’s iodine direct wet preparations were performed at the same time, one slide by using normal saline (0.85%) for demonstrating the trophozoites motility and a slide with Lugol’s iodine 5% for identification of internal protozoal structures [8].

**Blood Specimens Collection:** Five ml of blood was collected from each patient infected with *G. lamblia* and healthy person. Blood samples were drawn in sterile plain tubes and were left for 30 minutes at room temperature. Then the tubes were centrifuged for 5 minutes at 3000 rpm. Serum was collected and kept in sterile tubes in deep freeze (-20) until use.

**Detection of Serological Markers:** Two human biomarkers (hormones) were measured in this study. The serum concentration of the two markers; ghrelin and obestatin (Elabscience Company, Bulgaria) was determined by using ELISA technique according to the manufacturer procedure.

**Statistical analysis:** Data was collected, summarized, analyzed and presented using and Microsoft Office Excel 2010 and statistical package for social sciences (SPSS) version 23. The comparisons between the difference in mean concentrations of ghrelin or obestatin of the giardiasis patients and the healthy control persons were analyzed by T-test. And Spearman correlation was used to evaluate the correlation between the concentrations of ghrelin and obestatin and the results were expressed as the correlation coefficient (r). P-value less than 0.05 was considered statistically significant.

**Results**

The current study enrolled a total of 48 patients (28 males and 20 females) with *Giardia lamblia* infection and 48 healthy persons (26 males and 22 females). Overall, the age range was from 2 years up to 71 years and 2 years up to 70 years for patients and healthy persons (control group) respectively.

The obestatin serum level of patients with giardiasis and healthy persons
The serum concentration of obestatin, in terms of mean ± standard deviation, were 186.53 ±1.56 ng/ml and 92.88 ±2.9 ng/ml in all patients with giardiasis and healthy persons respectively; with a highly significant difference (p = < 0.0001), 186.71 ±1.6 ng/ml and 93.307 ±3.1ng/ml in male patients with giardiasis and male healthy persons respectively; with a high significant difference (p = < 0.0001) and 186.28 ±1.5 ng/ml and 92.157 ±2.6 ng/ml in female patients with giardiasis and female healthy persons respectively; with a high significant difference (p = < 0.0001) as shown in figure 1.

The ghrelin serum level of patients with giardiasis and healthy persons

The serum concentration of ghrelin, in terms of mean ± standard deviation, were 28.848 ±3.2 ng/ml and 81.387 ±3.8 ng/ml in all patients with giardiasis and healthy persons respectively; with a highly significant difference (p = < 0.0001), 28.885 ±3.2 ng/ml and 82.521 ±3.7ng/ml in male patients with giardiasis and male healthy persons respectively; with a highly significant difference (p = < 0.0001); and 28.796 ±3.2 ng/ml and 79.800 ±3.9 ng/ml in female patients with giardiasis and female healthy persons respectively; with a high significant difference (p = < 0.0001) as shown in figure 2.
3.3. The relationship between Serum concentration of Ghrelin and Obestatin

The present study revealed a significant positive association between the serum levels of ghrelin and obestatin in *G. lamblia* infected persons \( r = 0.0954 \) (Figure 3).

![Graph showing the association between ghrelin and obestatin serum levels in *Giardia lamblia* infected persons.](image)

\[ y = 0.6296x - 88.582 \]
\[ R^2 = 0.0954 \]

**Fig. (3). The association between ghrelin and obestatin serum levels in *Giardia lamblia* infected persons.**

**Discussion**

*Giardia lamblia* is an intestinal protozoan and distributes throughout the world. In 2013, the infectivity rate of giardiasis in Iraq was 1.77% and the highest rate was reported in Najaf province which was 7.9% \[^9\]. So, *G. lamblia* infection is considered as a significant health problem in Najaf province.

The current study revealed a significantly lower serum level of ghrelin in patients with giardiasis in comparison with the healthy persons. The results of this study agree with the study of Al-Hadraawy *et al.* \[^5\] Which proved the decrease of serum ghrelin level patients with giardiasis. In addition, the current study agrees with most studies which proved the decrease of ghrelin concentration with parasitic disease, as in the study of Ernsoy *et al.* \[^4\].

Ghrelin level reduced in patients with *G. lamblia* infection and other protozoal and helminthic infections are suspected to recompense for a raise in the serum sugar level in these infections; explained by the association between ghrelin and insulin \[^10\]. The reduced ghrelin level in these patients recorded in the current study is consistent with the suggestion that this may be the important cause of the anorexia in patients with giardiasis \[^11\], also the loss of appetite could be the main cause that leads to loss of weight in giardiasis patients.

This decrease of the ghrelin may be due to the intestinal hypermotility in patients with giardiasis \[^11\] because the protozoan *G. lamblia* colonizes in the duodenum and jejunum by attaching to the intestinal epithelium but without any mucosal invasion \[^12\]. The mechanism of pathogenesis is summarized by the destruction of the small intestine mucosal barrier, resulting in inflammation with malabsorption of fats \[^13\].

Another probable reason which can interpret, at least partially, the low serum ghrelin concentrations in persons with giardiasis might be to reduce the peroxidation of lipid that elevated as a consequence of this protozoal infection \[^6\].

The results of the current study revealed a significantly higher serum levels of obestatin in patients with giardiasis in comparison to the healthy persons, this increase could be due to parasitic damage to mucosa of intestinal in the acute period of infection such as inflammatory, ulcerative, and pathological changes in the epithelial cells villi \[^14\]. Indeed, none of the reviewed articles study the correlation between giardiasis and serum concentration of obestatin.
This high level of obestatin is suspected to be one of the important causes beside the low level of ghrelin (as above) for the appetite loss in *G. lamblia* infected persons. The increased level of obestatin and decrease level of ghrelin reported in the current study supports what is reported by Lacquaniti et al., [7] Who stated that: opposite to ghrelin, obestatin reveals as an appetite stimulant (anorectic) hormone, reducing intake of food, decreasing the emptying of the stomach and motility of jejunum, and decreasing gain of weight, and the study of Pan et al., [15] who reported that the effect of obestatin on food intake is in contrast to ghrelin.

This difference in ghrelin and obestatin levels may be due to the gastric infection that associated with the severity of chronic inflammation and glandular atrophy in the corpus of the stomach [16].

The present study disagrees with the study of Tschöp et al., Which showed that the circulating levels of ghrelin are increased in anorexia, but obestatin is low as well as the giardia trophozoites causes an increase in the host disruption and intestinal motility [17].

In the current study, in spite of the rise in the obestatin serum concentration and the reduce in the ghrelin serum concentration in persons infected with giardiasis, the serum level of ghrelin correlated positively and significantly with the serum obestatin level. This may be explained by the fact that obestatin is a 23-amino acid peptide hormone that is derived from post-translational cleavage of preproghrelin, the same peptide precursor as ghrelin, which is a 28-amino acid peptide released from the stomach [7]. Ghrelin, a polypeptide hormone which increases appetite, and the results of this research revealed decreased levels in giardiasis. Obestatin, as an anorexigenic hormone is an important factor to fit into the complexity of the pathophysiological scenario of the protein energy wasting [18].

Beside loss of appetite and nausea, loss of weight and failure to thrive in children may be caused by the inhibition action of obestatin on ghrelin stimulation of growth hormone levels [19], where the ghrelin is able to promote adipogenesis in the adipose tissue by directly stimulating the growth hormone secretion in the pituitary and on energy-regulating centers in the hypothalamus [20] and by making a communication between the brain and the gastrointestinal tract [21].

**CONCLUSIONS**

This study concluded that ghrelin and obestatin are important physiological biomarkers can support the diagnosis of *Giardia lamblia* parasitic infection. In addition, the elevation in the serum level of obestatin, and the decrease in the level of ghrelin may be the important cause of the anorexia, loss of weight and failure to grow in patients with giardiasis.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

All procedures were performed in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The Kufa Medical College Ethical Committee approved the protocol of this study. Informed consent was obtained for each patient and healthy person. The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


https://doi.org/10.1080/13102818.2016.1149038


Evaluation of the Efficacy of Beauveria bassiana in Controlling the Periplaneta Americana in Iraq

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Abstract

The current study was conducted from the ninth month of the year 2019 to the end of the sixth month of 2020 aiming and evaluating the effectiveness of the fungus Beauveria bassiana in controlling the Periplaneta americana.

More than 250 samples were collected, and the most of them were collected for the purpose of studying Biology control. The fungus Beauveria bassiana was used in the control, where the fungus was used in four different concentrations and kneaded by mixing with water and dilution, where the concentrations were used (0.25 × 107, 0.5 × 107, 0.75 × 107, 1 × 107) and all recorded death rates of 100% in nymphs after the passage of (244,168,120,96) respectively, and in adults, a murder rate was recorded after (244,192,144,120) hours respectively, and there were significant differences in the percentage of homicide between adults and nymphs.

Keywords: Periplaneta americana, Beauveria bassiana, Cockroaches

Introduction

Cockroaches have survived on Earth for more than 300 million years, almost without change. There are approximately 3,500 species of cockroaches around the world, 50 of them are considered household pests, and the remains of their food secrete foul-smelling secretions in their places, but they are not pests. Therefore, it is dangerous, it is found in abundance near areas where there is often stagnant water or areas where there is usually constant humidity such as toilets, kitchens and sewage, and cockroaches often feed on human faeces, garbage and sewage. Cockroaches cause allergies in children and transmit pathogens such as asthma, diarrhea, leprosy and polio (1,2,3).

The cockroach is one of the most common pests in city environments which is closely related to food that carries and spreads highly antimicrobial bacteria and can act as a vector for many microorganisms that affect public health (4). Although the cockroach is not the most important vector of disease, but it has a role in transmitting some diseases such as allergies as well as the spread of diseases such as: cholera, leprosy, dysentery, diarrheal diseases and plague, cockroaches carry filth and pathogens on their legs and bodies and contamination of food upon contact (5,6).

The control of cockroach groups depends on the control of insecticides, which are among the oldest methods, such as the use of pyrethroids, organophosphates, organic chlorine, carbamates, and stomach toxins, such as hydramethlonone and sulfuramide (7).

Entomopathogenic fungi has been in use for several years as a biological control tool for many insect pests as alternatives or supplements to chemical pesticides (8). Among these species is Buveara bassiana, as commercial preparations of this fungus have proven successful in controlling many pests, as insects infect a disease known as White muscardin (9).

The aims of the current study:

1- Evaluation of the efficiency of Buveara bassiana in controlling cockroaches.

3- Evaluation of the efficiency of Buveara bassiana in controlling the Periplaneta Americana in Iraq.
Materials and working Methods

Cockroach collection and breeding

Male and female cockroaches were collected from three different areas of the city of Samarra, namely Al-Mu’tasim sub-district, Al-Huwaish district, and the city center of Samarra. 300 cockroaches were collected through the net and hand traps, and by using chemicals to push the cockroach out of the sewage streams, insects were raised and multiplied in plastic containers with dimensions 25 x 40 A poison in which wood structures were placed to accommodate the cockroach in the form of tunnels were covered with a layer of dull cloth (10).

It was placed inside a room in a dark place with the provision of appropriate conditions, from a temperature ranging between 25-45 °C and a relative humidity of 65-70%. The appropriate note is to change the water and food every ten days to avoid rotting the bread (11).

The source of the fungus B. bassiana: -

The fungus was obtained from the Ministry of Agriculture / Plant Protection Department, the biotechnology project as a commercial product, and it was used according to the instructions issued by the Department of Prevention by dissolving (5) grams of the powder in a liter of distilled water, after which the dilution process was carried out to concentration by adding distilled water to Main focus (Department of Plant Protection, 2019).

Control of Periplaneta americana and nymphs:

The mushrooms were cultivated on an agricultural medium to ensure the effectiveness of the fungus. Potato agar was used as an agricultural medium for the fungus. Five adult insects were placed in transparent plastic containers and three replications were made. The insects were sprayed with different mushroom concentrations for each container and the concentrations were 0.25 x \(7^{10}\), 0.5 x \(7^{10}\) x 0.75 x \(7^{10}\), 1 x \(7^{10}\) and with three replicates for each concentration. The control repeater was sprayed with distilled water only. The containers were placed in an incubator on a temperature of 2 ± 28 °C and a relative humidity of 5 ± 70% to maintain the water content of the cockroaches and mushrooms. The insects were monitored for a period of ten days, and the results were recorded every 12 hours.

Microbial control with B. bassiana of adults and nymphs Periplaneta americana.

The results of Table (1) show that the highest concentration of killing is the concentration 1 x \(7^{10}\), and the lowest concentration in terms of the killing rate is the concentration of 0.25 x \(7^{10}\). The concentration of 1 x \(7^{10}\) gave the highest percentage of killing after passing 48 hours, at a rate of 0 6%, followed by a concentration of 0.75 x \(7^{10}\) spore / ml, at a rate of killing 40%, then a concentration of 0.5 x \(7^{10}\) spore / ml, and the concentrations were recorded at 0.5 x \(7^{10}\), 0.75 x \(7^{10}\), 1 x \(7^{10}\) spore / ml, the rate of killing after The passage of 72 hours (80,60,40)%, respectively. After the passage of 96 hours, the concentrations were recorded (0.25 x \(7^{10}\), 0.5 x \(7^{10}\), 0.75 x \(7^{10}\), 1 x \(7^{10}\)) spore / ml killing rate (100,80,40,20)%. Respectively, after 120 hours, the two concentrations (0.25 x \(7^{10}\), 0.5 x \(7^{10}\)) were recorded as a spore / ml killing percentage (80.60)%, respectively, and the concentration reached 0.5 x \(7^{10}\) to a 100 killed percentage after 168 hours and the concentration reached 0.25 x \(7^{10}\) spore / ml to 100% killing after 240 hours.
Table (1) of the effects of the fungus B. bassiana, whole Periplaneta americana nymphs.

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>Time / hour</th>
<th>Average focus</th>
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<tbody>
<tr>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>B. bassiana fung</td>
<td></td>
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</tr>
<tr>
<td>0.25×710</td>
<td>0.0</td>
<td>20</td>
</tr>
<tr>
<td>0.5×710</td>
<td>0.0</td>
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<tr>
<td>0.75×710</td>
<td>0.0</td>
<td>40</td>
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<tr>
<td>1×710</td>
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<td>60</td>
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Average killing for an Periplaneta americana B. bassiana

<table>
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<tr>
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<th>Time / hour</th>
<th>Average focus</th>
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<tr>
<td></td>
<td>5.0</td>
<td>22.5</td>
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Average general killing of an Periplaneta americana

<table>
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<th>Time / hour</th>
<th>Average focus</th>
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<tbody>
<tr>
<td></td>
<td>5.0</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>c</td>
</tr>
</tbody>
</table>

* Small horizontally similar letters mean no significant differences between them

* Similar vertically large letters mean no significant differences between them

Table (2) shows the effect of the fungus on Periplaneta americana adults in all concentrations, where the highest concentration of 1 × 710 spore / ml was recorded, a 40% killing rate after 48 hours, and a concentration of 0.25 × 710 spore / ml, a 20% killing rate, after 72 hours. The concentrations recorded (0.25 × 710, 0.5 × 710, 0.75 × 710, 1 × 710) spore / ml killed a percentage of (60,60,20,20)%, respectively, but after 96 hours the concentration was recorded (0.5 × 710, 0.75 × 710, 1 × 710) spore / ml killing rate 80,80,60%, respectively, but after 120 hours the concentrations were recorded (0.5 × 710, 0.75 × 710, 1 × 710) spore / ml killing percentage) 100, (80.40)%, respectively, and after 144 hours, the two concentrations (0.25 × 710, 0.5 × 710) recorded a spore / ml killing percentage (100,80) respectively, and the concentration reached...
Table (2) of the effects of the fungus B. bassiana, whole *Periplaneta americana* adults.

<table>
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<th>Extract concentration</th>
<th>Time / hour</th>
<th>Average focus</th>
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<tbody>
<tr>
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<tr>
<td>0.25 x 10^7</td>
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<tr>
<td>0.5 x 10^7</td>
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</tr>
<tr>
<td>0.75 x 10^7</td>
<td>0.0</td>
<td>20</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>0.0</td>
<td>40</td>
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<table>
<thead>
<tr>
<th>Average killing for an <em>Periplaneta americana</em> B. bassiana</th>
<th>0.0</th>
<th>0.0</th>
<th>20.0</th>
<th>25.0</th>
<th>0.0</th>
<th>60.0</th>
<th>60.0</th>
<th>38.8</th>
<th>22.5</th>
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<tr>
<td></td>
<td>eA</td>
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<td>dB</td>
<td>cA</td>
<td>eB</td>
<td>aA</td>
<td>aA</td>
<td>bB</td>
<td>cB</td>
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<tr>
<td>Average general killing of an <em>Periplaneta americana</em></td>
<td>5.0</td>
<td>0.0</td>
<td>0.0</td>
<td>22.5</td>
<td>0.0</td>
<td>12.5</td>
<td>40.0</td>
<td>52.5</td>
<td>54.4</td>
<td>26.3</td>
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<td></td>
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* Small horizontally similar letters mean no significant differences between them

* Similar vertically large letters mean no significant differences between them

(0.5 x 10^7) spore / ml to 100% killing after 192 hours. As for the concentration (0.25 x 10^7) spore / ml, it reached a 100% killing rate after 240 hours. It was found through the results of the study that the nymphs were more susceptible than the whole insects to the fungus, and this is due to the frequent shedding that causes the nymph to undergo periods of weakness in the Q-layer in the initial stages after molting, which facilitates the process of penetration by the hepatitis of B. bassiana. It was also found that the effect of reducing the concentration of the fungal suspension was significant on the severity of the infection, and that the correlation was positive between the percentage of killing and the concentration of the sporophyte suspensions of the fungus, and the dilution could be considered an important factor in the efficiency of the fungus in eliminating the *Periplaneta americana*.

(12)

The study showed that increasing the duration of exposure is another determinant factor for increasing the efficiency of the fungus so that the spores can grow and begin to penetrate the cockroach’s qi and penetrate into the internal tissues of the cockroach, as well as making holes in the q-layer and thus increasing the chance of the cockroach being exposed to water loss, and the direct influence of environmental conditions as a result of exposure to the body wall. To the damage caused by the penetration of the hyacinths of the fungus to the wall of the body. (13)

The results of this study gave clear evidence of the possibility of using the fungus B. bassiana in controlling and eliminating the *Periplaneta americana* under specific conditions and times that depend on moderate temperatures and suitable humidity, which is a prerequisite for the control procedure, because the fungus needs specific conditions of temperature and humidity to enable it to activate its efficiency in infestation. The insect and its elimination. (14)
pointed out about the effect of fungi on the life roles of mosquitoes, as the fungus succeeded in controlling mosquitoes as well as succeeding in controlling the spread of malaria.

Conclusions

The possibility of using microbial control to reduce the spread Periplaneta Americana.

Fung B. bassiana can be used to control the spread of the *Periplaneta Americana*.

Recommendations

Conducting further studies using microbial control to reduce cockroach spread

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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The Effect of Biomarker (IL-8) on the Tissue of Colorectal Cancer Patients by using IHC Technique

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Abstract

Aim: The search to evidence a link between the molecular markers (IL-8) with colorectal cancer. The aim of the presented work is examining the relation regarding the biomarker (IL-8) in colorectal cancer (CRC) tissues as well as its relation with the other clinic pathological variables.

Methods: The detection of IL-8 was confirmed using histopathological examination of colonic biopsy of tissue section fixed on slides previously coated and analyzed by immunohistochemistry method (IHC), hence to detect the variable regarding IL-8 expression in normal as well as CRC tissues including (adjacent normal tissue and healthy controls tissues); in addition to its association with other clinic-pathological variables (intensity, gender and age).

The results of the study specified the enrichment regarding IL-8 expression in the samples of CRC with the use of IHC approach. No considerable relation has been indicated between the expression of IL-8 with the other clinical-pathological variables (gender and age), yet there has been considerable relation between the expression of IL-8 and the stain’s intensity (grade), while it has been increased in tissues of CRC; this might be specified as one of the risk factors with regard to the CRC’s development and the metastasis in the samples of CRC, also it might be significant marker to predict insignificant prognosis in patients experiencing CRC, also it can be utilized as possible therapeutic target in the CRC.

Keywords: Immunohistochemistry technique, Biomarker, Colorectal cancer, Metastasis.

Introduction

The cancer’s incidence is increased yearly. CRC can be considered as the second most general cause regarding mortality from cancer in Western world ¹. A lot of factors (genetic and environmental) have been involved in mortality as well as propagation resulted from CRC. From the many trophic factors, chemokines were of major role.

IL-8 can be defined as chemokine that is created through a lot of tumor in addition to normal cells, also its major role is in the amplification and initiation regarding acute inflammatory reactions. Furthermore, IL-8 was involved in the chronic inflammatory process in addition to the diseases with the chronic inflammatory component including cancer (colorectal), the level has been increased with the progression and metastasis of disease ²,³.

Interleukin-8 (IL8), belong to neutrophil-specific CXC sub-family of chemokine (ELR) with defining CXC amino acid motif including (Leu, Arg, and Glu), which might not only be acting on Leukocyte chemotaxis, infectious diseases and inflammatory responses ⁴. IL8 is generated by many different normal cells and tumor cells and its main goal is initiating and amplifying acute inflammatory reactions. IL8 was implicated as well in the chronic inflammatory diseases and procedures with chronic inflammatory components like the cancer ³. It has been known to be possessing pro-angiogenic and tumorigenic characteristics. IL8 over-expression was discovered in numerous human tumours, inclusive the CRC, and is related to insufficient prognoses ⁵. The cells of the tumor generate IL8 as an autocrine factor.
of growth that promotes the growing of the tumor, metastatic spread, and invasion of tissues. Ning and coworkers reveal that the use of a xenograft model of the tumor, cells that express IL8 produced considerably larger tumors compared to control cells with enhanced density of the micro-vessels.

Whereas the applications related to the immunological approaches to the histopathology caused marked improvement in microscopic diagnosis that is related to the neoplasm. Even through that the histological analysis regarding Haematoxylin & Eosin (H&E) stained tissue section remain the core related to practice of neck and head surgical pathology, due to the fact that immunohistochemistry (IHC) remains the major applied and provided approach in pathology for determining the expression status regarding tumor-associated proteins, also for studying the clinical prognostic relevance related to the biomarkers.

This work has been developed for examining the differences in the expression of IL-8 between the tissues of CRC in addition to the matched adjacent normal mucosa tissues, as well as the relation between expressions of IL-8 as well as the clinic pathological features in the CRC.

Materials and Methods

Surgical specimens

Fresh specimens (80) of colorectal tissues were collected from patients with CRC (n=42) beside healthy control individuals (n=10). Likewise; (n=28) CRC samples from (n=42) were matched with adjacent normal tissue (n=28). All these specimens were obtained from patients underwent colonoscopy and segmental colonic resections, those that were diagnosed as having colorectal cancer with different grades or healthy tissues were considered as a control group.

All these tissue samples were collected from patients; admitted maintained in different hospitals in Baghdad city, during the period of 2018 -2019.

All sought after datum on demographic and clinical histopathological parameters which was gained from the patient’s medical records and designed information sheet. The exclusion criteria were on patients for CRC patients including chemotherapy or radiation treatments prior surgery, also excluded the patients who had another malignancies or polyps in another organs. All participants received conventional bowel preparation without preoperative antibiotic administration.

Histopathological Examination

It was performed according to the method. The histopathological examination of the colon tissues which were selected from the patients of CRC were determined after fixing, and sectioning the organs, then staining them as well as Immunohistostaining. All these were performed in the Educational Laboratories in the City of Medicine as follows:

1- Preparation of Histological Sections:

At the moment of eradication the part of Colon tissue or Rectum was removed and then washed with PBS, preserved and fixed in 10% formalin for (24-48) hr. Then the section was washed up with tap water and processed with a set of increasing ethanol concentrations for 2hr at each concentration. After that, the tissue was clarified in xylene for 2hr, then it was impregnated in melting paraffin at 60-70°C for 1hr. The tissue was embedded in paraffin blocks and waited to be solidified, finally it was sectioned using a microtome at slices of (5-6μm) and they were mounted on the slides. The sections of tissue have been stained with the eosin stains and hematoxylin, after that the cell morphology has been identified within light microscopy.

2- Immunohistochemical examination:

Specimen preparation for IHC staining:

The tissue sections of human cancer subjected to immunohistochemistry analysis with the use of GenTex System (Gen Tex / USA) as well as PolyExcel HRP/DAB Detection System (Pathinsitu / USA) on the basis of the instructions of manufacturer. Put briefly, the tumor blocks have been formalin-fixed, paraffin-embedded and cut into 4-μm-thick sections.

Antibody dilutions:

IL-8 is concentrated antibodies, hence diluted them with antibodies diluent according to company (Pathinsitu and Gen Tex) sheet enclosed with them:

IL-8 was diluted by ratio of 1:100-1:1000 (according
to the sheet between 100-1000), by taking 1xPBS, 20%Glycerol(pH7), 0.025% ProClin 300 which was added as a preservative.

Controls of marker:

Tissues of Gastric Ca known to express the antigens of interest have been utilized as positive controls. Whereas for negative control, omitting primary antibody and after that has been replaced with PBS.

Immunohistostaining steps:

This was accomplished according to 10.

The slide’s evaluation has been made through the pathologists which is blinded for the characteristics of patients. Slides have been scored in the following way on the basis of an approach suggested via 11: Intensity related to staining has been classified from 0 to 3, as 0(-) negative, 1(+) low or weak, 2(++) moderate, and 3(+++) high or strong. The staining’s extent has been indicated as the percentage regarding positive cells with regard to the whole tumor area, has been classified from 0 to 3, as 0(0%), 1(10-20%), 2(20-50%), 3(>50%). The final staining score has been evaluated through adding staining intensity as well as the staining’s extent.

Statistical Analysis

Data analysis has been conducted with the use of available statistical package of SPSS-25. The data have been provided in simple frequency measure, percentage, standard deviation, mean, in addition to range (minimum-maximum values).

The significance of difference regarding various percentages (qualitative data) has been tested with the use of Pearson Chi-square test ($c^2$-test) with application regarding Yate’s correction or Fisher Exact test when appropriate. Statistical significance has been specified when the P value has been equal or not more than 0.05.

Results

H&E stained

The results of intensity of the stains (grade of differentiation) was classified from 0 to 3, as 0(-) negative, 1(+) poorly, 2(++) moderate, and 3(+++) well differentiated.

The expression of IL-8 in CRC

The results regarding cytokine IL-8 illustrated an increased in IL-8 expression [from score (+1, +2, +3)] as seen in (Figures 1A,1B, and1C) respectively, as well as positive stained cells in cancer cells of patient compared with healthy control, and adjacent normal tissue. The results of statistical analysis indicated a significantly increased in IL-8 expression in CRC tissue in comparison to adjacent controls as well as healthy control individuals as showed in (Tables 1, and 2); while no significant association appeared between adjacent normal tissue compared with healthy control tissue (Table 3). As well as no considerable relation has been indicated between IL-8 expression and other clinic pathological variables (age, gender) (Table 4).

Figure 1: IL-8 IHC Scores expression (400x): A- Score +1 expression; B- Score +2 expressions; and C- Score +3 expressions.
Table 1: The data regarding the significant expression of IL-8 scoring and intensity of stain in patient’s cancer tissue compared to healthy control individual’s tissue.

<table>
<thead>
<tr>
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<th>Patient “Cancer tissue” (n=42)</th>
<th>Healthy control (n=10)</th>
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<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>IL-8 Score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[0]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[+1]</td>
<td>2</td>
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<tr>
<td>[+2]</td>
<td>13</td>
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</tr>
<tr>
<td>[+3]</td>
<td>27</td>
<td>64.3</td>
</tr>
<tr>
<td>P value compared to healthy control tissue</td>
<td>0.0001*</td>
<td>-</td>
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</table>

| IL-8 Intensity|    |     |    |     |
|---------------|    |     |    |     |
| [0]           |  - | -   | 8  | 80.0|
| [+ ]          | 9  | 21.4| 2  | 20.0|
| [++]          | 17 | 40.5| -  | -   |
| [+++]         | 16 | 38.1| -  | -   |
| P value compared to patient normal tissue | 0.0001* | - |

*Significant difference between proportions using Pearson Chi-square test at 0.05 level.

Table 2: The data regarding the significant expression of IL-8 scoring and intensity in patient’s cancer tissue compare to patient’s adjacent tissue

<table>
<thead>
<tr>
<th></th>
<th>Patient “Cancer tissue” (n=28)</th>
<th>Patient “Adjacent normal tissue” (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>IL-8 Score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[0]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[+1]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[+2]</td>
<td>11</td>
<td>39.3</td>
</tr>
<tr>
<td>[+3]</td>
<td>17</td>
<td>60.7</td>
</tr>
<tr>
<td>P value compared to patient adjacent tissue</td>
<td>0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

| IL-8 Intensity|    |     |    |     |
|---------------|    |     |    |     |
| [0]           |  - | -   | 14 | 50.0|
| [+ ]          | 6  | 21.4| 12 | 42.9|
| [++]          | 13 | 46.4| 2  | 7.1 |
| [+++]         | 9  | 32.1| -  | -   |
| P value compared to patient adjacent tissue | 0.0001* |   |

*Significant difference between proportions using Pearson Chi-square test at 0.05 level.
Table 3: The data regarding of no significant association of expression IL-8 scoring and intensity between the adjacent normal tissue of the patients and healthy control individuals

<table>
<thead>
<tr>
<th>Patient “Adjacent normal tissue” (n=28)</th>
<th>Healthy control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>[0]</td>
<td>14</td>
</tr>
<tr>
<td>[+1]</td>
<td>9</td>
</tr>
<tr>
<td>[+2]</td>
<td>5</td>
</tr>
<tr>
<td>[+3]</td>
<td>-</td>
</tr>
<tr>
<td><strong>P value compared to healthy control tissue</strong></td>
<td>0.191</td>
</tr>
</tbody>
</table>

Table 4: The data regarding no significant association between the expressions of IL-8 scoring compared with age and gender

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>IL-8 for Patient “Cancer tissue” (n=42)</th>
<th>IL-8 for Healthy controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[0&amp;+1]</td>
<td>[+2]</td>
</tr>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>&lt;50y</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>50---59</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>60---69</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>=&gt;70y</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.696</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>IL-8 for Patient “Cancer tissue” (n=42)</td>
<td>IL-8 for Healthy controls (n=10)</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.866</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The over-expression regarding IL-8 was recognized in various human tumors, such as CRC, also it is related with the poor prognosis.5

With regard to colonic mucosa, the upregulation that is related to IL-8 happen in relation to the inflammation’s degree in ulcerative colitis and the Crohn’s disease.12,13 With regard to the human colon cancer cell lines, constitutive expression related to IL-8 was associated to the metastatic potential,14 also was indicated to be of high importance in the progression of distant metastases. Some researches indicated that the induction related to the IL-8 signaling elevated the NF-κB transcriptional activity.15,16 A study conducted via 17 examined the patterns related to the gene’s expression in colon cancer cells in addition to the corresponding normal mucous cells through DNA microarray analysis, indicating an elevation in the IL-8 expression in cancer cells in comparison to adjacent normal mucosa. Furthermore, a study by 18 indicated that increased levels of IL-8 mRNA in tumor tissue in comparison to corresponding normal mucous tissue, more often positive staining with regard to IL-8 protein specified in poorly and moderately differentiated tumors in comparison to excellently differentiated tumors (P=0.024). A research carried out via 14 assumed that IL-8 expression in colon carcinoma cells is of high importance in metastasis and growth, also such data in accordance with the results of 19. Results specified no considerable relation between IL-8 expression scoring a gender or age related to the patients.

While another supported that CXCL8 expression has been considerably upregulated in the tumoral samples in comparison with the normal tissue, and such upregulation elevated with the age of patients.2 The results suggested that IL-8 staining has been more common in well-differential and moderate tumors in comparison to adjacent and healthy controls which were stained poorly or mildly and negative differentiated. While Čačev et al.18 showed that the positive immunohistochemically IL-8 staining has been more common in poorly and moderately differentiated tumors in comparison to excellently differentiated ones.

Conclusion

The highly elevated expression of IL-8 in CRC tissues; may be specified as risk factor with regard to the CRC’s development and metastasis in CRC samples and may be of high importance marker to predict poor prognosis in the patients experiencing CRC that might be applied as possible therapeutic target in CRC.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References


Prevalence and Risk of Leukemia Reported Cases, Observational Descriptive Statistic from Iraqi Center for Hematology in Baghdad Province

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Abstract

Background: Leukemia is one type of blood cancer developed in the bone marrow and other blood-forming organs. Based on a combination of onset speed and cell origin, leukemia can generally be divided into four subtypes: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), (chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML).

Methods: A total of 3102 eligible leukemia cases were documented in the Iraqi Center for Hematology in the City of Medicine in Baghdad between January 2018 and December 2019. 1402 documented in 2018 and 1700 in 2019 for all types of cancer. Patients ages were between 1 year and 90 years, among all leukemia cases, the mostly 36% (n = 730) were aged ≤15 years. Results In our study males accounted for a higher proportion of leukemia patients, 58% compared to 42% females, ALL was the more prevalent type among the studied group for both years; contribute% 55.17 din male %53.45 in female, followed by AML 36.87% vs 40.27%, CLL 5.23% vs 2.22% and CML 2.73% vs 4.06% in male and female respectively. Conclusion It was observed that the prevalence of leukemia in Baghdad increased between (2018-2019) from 1402 to 1700, the youngest age 15 registered most of leukemia cases. Males were mostly victim of the disease as compared to females which were less frequent. It was observed that acute lymphoblastic leukemia (ALL) was most common type of leukemia.

Key Words: Leukemia; Prevalence; acute myeloid leukemia; chronic lymphocytic leukemia; and chronic myelogenous leukemia.

Introduction

Leukemia is type of blood cancer originally developed in the bone marrow and other blood-forming organs. Based on a combination of onset speed and cell origin, leukemia can generally be divided into four subtypes (1).

(I) Acute lymphocytic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extramedullary sites; While 80% of ALL occurs in children, it represents a devastating disease when it occurs in adults. Within the United States, the incidence of ALL is estimated at 1.6 per 100,000 population. In 2016 alone, an estimated 6590 new cases were diagnosed, with over 1400 deaths due to ALL (American Cancer Society). The incidence of ALL follows a bimodal distribution, with the first peak occurring in childhood and a second peak occurring around the age of 50 (2).

(II) Acute myelogenous leukemia (AML) is the second most common pediatric leukemia with poor outcomes indicated by 5-year survival rates of 50-60% (3). The difficulty of obtaining bone marrow samples and the in vitro cytarabine chemo sensitivity in primary AML cells is probably the reason for the lack of such studies, especially in pediatric patients (4).
(III) Chronic lymphocytic leukemia (CLL) is characterized by a clonal expansion of mature CD5 CD23+ B-lymphocytes that accumulate in the bone marrow and infiltrate lymphoid tissues such as the spleen and lymph nodes (5). CLL is the most common leukemia in the Western world, is a heterogeneous disease remains incurable in virtually all cases. CLL predominates in the elderly, and the incidence of the disease increases exponentially with age (6). Thus, the number of CLL patients is expected to rise in the future, given the increase in the aging population, bringing to light new clinical challenges and public health issues. Patients with CLL show a tremendously variable clinical course ranging from excellent prognosis with no treatment to short-term survival, despite early initiation of therapy (7).

(IV) Chronic myelogenous leukemia (CML) Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm with an incidence of 1–2 cases per 100 000 adults. It accounts for approximately 15% of newly diagnosed cases of leukemia in adults. In 2017, it is estimated about 9000 new CML cases will be diagnosed in the United States, and about 1000 patients will die of CML. Since the introduction of imatinib in 2000, the annual mortality in CML has decreased from 10%-20% down to 1%-2%. Consequently, the prevalence of CML in the United States, estimated at about 25–30 000 in 2000, has increased to an estimated 80–100 000 in 2017, and will reach a plateau of about 180 000 cases by 2030 (8).

Finally cancer is one of the leading causes of mortality in developed and developing countries. It is expected that the Incidence and burden of cancer will increase throughout the World due to the population growth and aging especially in less developed countries which account for about 82% of the world’s population (9).

Material and Methods
We conducted a retrospective, descriptive, epidemiological study of leukemia cases for both gender in the Iraqi population diagnosed between January 2018 and December 2019. The data of this study that include the gender, clinical history, age, type of leukemia were managed and registered by Iraqi Center for Hematology, which is a clinical center in the City of Medicine in Baghdad, that diagnose and registry hematology disease in the Iraq except Basra and Kurdistan regions. The variables calculated were for example patient’s age, gender, type of leukemia and their residence addresses. Controls have been chosen by keeping the patients’ detail in consideration. Diagnoses were including peripheral blood films and morphology of bone marrow which involved cytochemical staining and immunophenotyping. Selected topic was accepted by scientific committee; official acceptance was taken from health authorities to conduct this study. Collected information was kept confidential.

Statistical Analysis
All statistical analysis was carried out by using statistical package for social sciences (SPSS) 16.0 version. Odds ratios with 95% confidence intervals were calculated to determine the strength of the associations.

Results
A total of 3102 eligible leukemia cases were documented in the Iraqi Center for Hematology between January 2018 and December 2019. 1402 documented in 2018, 900 were males (64.2%) and 502 were females (35.8%) and 1700 in 2019, 898 were males (52.8%) and 802 were females (47.2%). The percentage of cases with leukemia in male was more than female (Table 1). Patients ages were between 1 year and 90 years, among all leukemia cases, the mostly 36% (n = 730) were aged ≤15 years.

The commonly morphological distribution of leukemia in 2018 was acute lymphoblastic leukemia (ALL) by 498 cases (65%) for males and 273 cases (35%) for females followed by acute myeloid leukemia (AML) by 335 cases (63%) for males and 198 cases (37%) for females then chronic lymphocytic leukemia (CLL) by 59 cases (84.3%) for males and 11 cases (15.7%) for females and finally chronic myeloid leukemia (CML) by
8 cases (29%) for males and 20 cases (71%) for females (Table 2).

The commonly morphological distribution of leukemia in 2019 was acute lymphoblastic leukemia (ALL) by 494 cases (55%) for males and 424 cases (52.9%) for females followed by acute myeloid leukemia (AML) by 328 cases (36.5%) for males and 327 cases (40.8%) for females then chronic lymphocytic leukemia (CLL) by 35 cases (3.9%) for males and 11 cases (15.7%) for females and finally chronic myeloid leukemia (CML) by 8 cases (29%) for males and 20 cases (71%) for females (Table 3).

Regarding the distribution of types of leukemia in our study as a total, ALL was the more prevalent type among the studied group, contributed (55.17% male versus 53.45% female), followed by AML (36.87% male versus 40.27% female), CLL (5.23% male versus 2.22% female) and the less frequent type was CML which contributed only (2.73% male versus 4.06% female). The results show a significant difference in number of cases between male and female in all types. ALL, AML and CLL more incidence in male than female while CML more incidence in female than male. (Table 4).

Table 1: Gender Distribution and percentage of 3102 Cases of Leukemia Reported During the Period 2018 – 2019

<table>
<thead>
<tr>
<th>Gender</th>
<th>2018 Count (%)</th>
<th>2019 Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>900 (64.2)</td>
<td>898 (52.8)</td>
</tr>
<tr>
<td>Female</td>
<td>502 (35.8)</td>
<td>802 (47.2)</td>
</tr>
</tbody>
</table>

Table 2: Distribution of Leukemia in Iraq, 2018

<table>
<thead>
<tr>
<th>Type of leukemia</th>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>498</td>
<td>65</td>
<td>273</td>
<td>35</td>
</tr>
<tr>
<td>AML</td>
<td>335</td>
<td>63</td>
<td>198</td>
<td>37</td>
</tr>
<tr>
<td>CLL</td>
<td>59</td>
<td>84.3</td>
<td>11</td>
<td>15.7</td>
</tr>
<tr>
<td>CML</td>
<td>8</td>
<td>29</td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td>900</td>
<td>100</td>
<td>502</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Distribution of Leukemia in Iraq, 2019

<table>
<thead>
<tr>
<th>Type of leukemia</th>
<th>Male</th>
<th>%</th>
<th>Female</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>494</td>
<td>55</td>
<td>424</td>
<td>52.9</td>
</tr>
<tr>
<td>AML</td>
<td>328</td>
<td>36.5</td>
<td>327</td>
<td>40.8</td>
</tr>
<tr>
<td>CLL</td>
<td>35</td>
<td>3.9</td>
<td>18</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Table 4. Comparison of Male and Female Patients with Different Types of Leukemia during 2018-2019

<table>
<thead>
<tr>
<th>Leukemia type</th>
<th>Male group (n=1798) 58%</th>
<th>Female group (n=1304) 42%</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>OR</td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>992 (55.17)</td>
<td>697 (53.45)</td>
<td>1.07</td>
<td>(0.92-1.23)</td>
<td>0.34</td>
</tr>
<tr>
<td>AML</td>
<td>663 (36.87)</td>
<td>525 (40.27)</td>
<td>0.86</td>
<td>(0.74-1.00)</td>
<td>0.05</td>
</tr>
<tr>
<td>CLL</td>
<td>94 (5.23)</td>
<td>29 (2.22)</td>
<td>2.42</td>
<td>(1.58-3.70)</td>
<td>0.00</td>
</tr>
<tr>
<td>CML</td>
<td>49 (2.73)</td>
<td>53 (4.06)</td>
<td>0.66</td>
<td>(0.44-0.98)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Discussion:

The present study demonstrated increasing in the leukemia cases from 2018 to 2019. This is logical due to the large number of wars and crises that occur in Iraq. In the last 31 years (1980-2010), Iraq has seen three wars, (Iran-Iraq war, 1980-88), (Gulf War, 1990-91), and (Iraq War, 2003) as well as Economic sanctions (1990-2003), Sectarian war (2006-2007) and American occupation (2003-2010). These varieties of wars and crises have a negative impact on the people’s health. (10). Similarly (Alghamdi et al.’(2014) and Ghojogh et al.’ (2015) reported increasing in the incidence of leukemia cases in Saudi Arabia and Iran patients respectively (11, 12).

The present local study demonstrating that the percentage of cases with leukemia in male (58%) was more than in female (42%). Iraqi population in other region of Iraq, Karbala Province also reported a similar finding that accounted 58.2% for males and 41.8% for females between November 2011 to May 2018 (13), in Brazil, it is estimated the prevalence of leukemia in male more than female also (14). Saudi study performed in 2001-2008 also confirmed the result (15).

The most prevalence aged were ≤15 years was clearly obtained (15), while another Iraqi study in Karbala Province, showed the most prevalence aged were ≤10 years (13). As well as 60% of patients in United States were under 20 years of age (16).

In our study ALL was the more prevalent type among the studied group; contributed 54.31% (% 55.17 in male %53.45 in female, followed by AML 38.57% (36.87% vs 40.27%), CLL 3.73% (5.23% vs 2.22%) and the less frequent type was CML which contributed only 3.4% (2.73% vs 4.06%). This result was in similar with the data from studies in other region of Iraq, Karbala Province that ALL is the more prevalent type with 41% followed by CML 24.1%, AML 19.2% and CLL 15.7% (13) and Sulaymaniyah province ALL was the most common type of leukemia with 44% in all cases, CML was the second type with 20% of cases followed by CLL, 18% and AML, 17% (17).

In Croatia, the most common type of leukemia was CLL, which accounted for 42% of leukemia, followed by AML with 27%, ALL with 17% and CML with 14% (18). In Bangladesh, on the other hand, AML was the most prevalent hematological malignancy 28.3% followed by CML 18.2 %, ALL 14.1 % and CLL 3.7 % was the least common (19). In addition, Indian study seem to reinforce the result that ALL is the most prevalent type...
in leukemia (20).

**Conclusion**

It was observed that the prevalence of leukemia in Iraqi Center for Hematology in Baghdad Province increased between (2018 - 2019) from 1402 to 1700, the youngest age ≤ 15 registered most of leukemia cases. Males were mostly victim of the disease as compared to females which were less frequent. It was observed that acute lymphoblastic leukemia (ALL) was most common type of leukemia. Epidemiological study can play a vital role in understanding the occurrence and outcome of the disease.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


2016; 129(2).
Detection of Fungal Toxins Produced by Dermatophytes by using Thin Layer Chromatography

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Abstract
Dermatophyte fungi include a wide range of filamentous fungi that are pathogenic to humans including three superficial cutaneous genera such as ; Epidermophyton, Microsporum and Trichophyton. However, the aim of this study isolation and identification of fungi responsible of dermatophytosis and detection of the dermatophyte ability to produce mycotoxins. The results of this study showed that 49(69.01%) out of 71 specimens were gave positive results , while 22 (30.98%) were gave negative results by examination on 10%KOH and cultured on Sabouraud dextrose agar with cyclohexmaide. Out of 49 culture positive isolates, 31(63.3%) T. rubrum isolates was the most frequent etiological agent followed by 11( 22.4%) T. mentagrophytes ; 5(14.3%) M. canis and 2 (4.1%) Epidermophyton floccosum isolates. The use of the TLC method to detect of mycotoxins in chloroform extracts for 49 dermatophyte mycelia. It was found all dermatophyte isolates are able to produce fluorescent stains with different colors under UV light at 365 nm , also these stains may be act as a non-enzymatic virulence indicator (mycotoxins).

Keywords: E. floccosum , Dermatophytosis, TLC , Mycotoxins.

Introduction
Dermatophytes are anamorphic genera which includes Epidermophyton, Microsporum and Trichophyton , all of these genera cause superficial fungal infection called dermatophytosis. Dermatophytes are also characterized as keratinophilic fungi, as they infect the skin, hair, and nails of humans and animals. Usually, dermatophyte infections are limited only to the outer layer of the epidermis and also unable to penetrate the deep tissues of a healthy individual (1) . Dermatophytes are included Anthropophiles ( infect human) , zoophiles ( infect animals) and geophiles (soil dwelling), all of these subdivisions according to their natural habitat. Dermatophytes are characterized by the secretion of large quantities of analyzed enzymes such as lipase , elastase, keratinase, phospholipase and protease depending on the specificity of the different substrate , the pathogenicity of host tissues occurs (2) . Keratinase enzyme produced by Bacillus species (3),(4) Streptomyces (5) and also dermatophytes fungi (6) . The outer layer of the epidermis characterized by being rich in keratinous substance such as the skin, hair and nails , which is considered a favorite for the dermatophytes fungi. In addition to keratinase, protease is also regarded one of the most important enzymes produced by dermatophytes as a virulence factor(7). There are non-enzymatic virulence factor that are produced by dermatophytes fungi such as ; xanthomegnin is mycotoxin produced by T. rubrum, which is also produced from Penicillium and Aspergillus , whether in vitro or in vivo causing nephropathy and death in animals. The T. rubrum culture characterized by red pigmentation on the reverse side , this is explained by the production of xanthomegnin which is also noted in infected skin and nail specimens (8). There are limited species of dermatophytes that have the ability to produce
 materials or melanin-like compounds, whether in vitro or in vivo, which play a similar role in pathogenesis of dermatophytic diseases (9). Aim of this study; isolation and identification of fungi responsible of dermatophytosis and detection of dermatophyte ability to produce mycotoxins such as Aflatoxin-like compounds chemically and separation of these mycotoxins by Thin Layer Chromatography (TLC).

**Materials and Methods**

**Isolation and Identification of fungal isolates:**
71 samples were collected from patients with dermatomycosis infections during period from February 2018 to January 2019. All the specimens were divided into two portions, one portion for direct examination under light microscope and other was cultured on sabouraud dextrose agar.

**Detection of dermatophytes ability to produce mycotoxins:**

**Isolation and purification of mycotoxins:**
We used nutrient broth (NB) to detection of mycotoxins produced by dermatophyte fungi. Subsequently, prepared 25 flask with volume 500ml then each flask added to it 250 ml NB, after sterilization and cooling, the culture media was inoculated with agar blocks of 5 mm of pure isolates grown on sabouraud dextrose agar SDA for 7 days at a rate of one disk per flask, except one flask left without inoculation as a control for comparison, and incubated at 28 °C for three weeks. After incubation, the entire contents were filtered with a sterile, clean gauze and then chloroform was added to the broth (1:1) in a separation funnel. The mixture was shaken for a few minutes then separated an upper layer containing spores and mycelia, and a lower layer containing chloroform and mycotoxins. The bottom layer filtered through a Whatman No. 1 filter paper then concentrated by using reflective condenser to approximately 1 ml in dark bottles (10).

**Detection of mycotoxin by using Thin Layer Chromatography technique (TLC):**

This experiment was carried out in a toxicology laboratory at the College of Applied Medical Sciences/ Karbala University, where used Thin Layer Chromatography plates (TLC) with dimensions of 20 * 20 cm, after activated in the electric oven at a temperature of 105 °C for an hour before use (11). The separation system used consists of chloroform : methanol 98:2 and 15μl of standard mycotoxin was taken by capillary tube, and put on the line a distance of 2 cm from left edge of the plate and at a distance of 2 cm between the spot of standard mycotoxin put an amount equal to the standard mycotoxin from the first fungal isolate extract and thus to the rest isolates extract. After that, the plate was placed in the separation tank containing the separation system consisting of a mixture of chloroform and methanol, at a ratio of 98: 2 v/v. The separation solution was monitored until it reached a distance of approximately 2 cm from the top end of the plate, then plates removed and dried under laboratory conditions for a period of 5min (12). Then compare the RF value and color for the mycotoxins spots with standard.

**Determination of UV absorbance:**

The partially purified mycotoxin compounds which produced by dermatophytes fungi subjected to UV radiation at a wavelength of 365 nm absorption spectrum on TLC to distinguish the absorption bands in the sample (12).

**Results and Discussion**

The result of this study showed 49(69.01%) out of 71 specimens were gave positive results, while 22 (30.98%) were gave negative results by examination on 10%KOH and cultured on Sabouraud dextrose agar with cyclohexmaide (SDAC) (Fig-1).
The results of this study showed that there is a statistical significant (P<0.05). This result is identical to the results of Lafta [13]; Maluki and Alaa [14]. Out of 49 culture positive isolates, 31(63.3%) T. rubrum isolates was the most frequent etiological agent followed by 11(22.4%) T. mentagrophytes; 5(14.3%) M. canis and 2(4.1%) Epidermophyton floccosum isolates (Fig-2).

The results of this study showed that there is a statistical significant (P<0.05). However, this result is matching with the results of Al-Shamei [15] but not compatible with results of Hindy and Abiess [16] who found Trichophyton species was most frequent etiological agent followed by Microsporum species. Anupama, [17] who found 33/63(52.4%) T. rubrum isolates were most predominant followed by T. mentagrophytes 20/63(31.7%) and M. gypseum 2/63(3.2%). This study showed that 49(100%) culture positive isolates possess the ability to produce fluorescent spots with different colors when chemically analyzed by using TLC technique. As well as, the fungal isolates varied in their production for these stains, depending on comparison of spots size and fluorescent intensity under UV light with standard mycotoxin as shown in (Fig-3).
The results of current study also showed that some of these spots had Rf. an identical to the standard Rf., while the other spots had Rf. different from the standard Rf. Although there are no previous studies and scarcity of references about this topic for the purpose of comparison, the precise diagnosis for these compounds is focus of the current studies, in our lab.

**Conclusion**

The use of the TLC method to detect of mycotoxins in chloroform extracts for 49 dermatophyte mycelia. It was found all dermatophyte isolates are able to produce fluorescent stains with different colors under UV light at 365 nm, also these stains may be act as a non-enzymatic virulence indicator (mycotoxins).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


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Possible Protective Effects of high- versus low- dose of lutein in combination with irinotecan on Liver of Rats: Role of Oxidative Stress and Apoptosis

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Abstract

Objectives: The current study was designed to describe roles of oxidative stress via the measurement of malondialdehyde and total antioxidant markers, and apoptosis through the measurement of caspase 3 marker, as mechanisms of liver toxicity induced by irinotecan; and to explore the possible protective effects of high- and low- doses of lutein against irinotecan induced toxicity in the liver of rats.

Methods: Thirty six (36) Sprague-Dawley rats were randomly divided into six groups: Groups I, rats received single oral daily dose of dimethyl sulfoxide (4 ml/kg); Group II (irinotecan-treated), received single oral daily dose of dimethyl sulfoxide (4ml/kg) for 25 days by oral gavage and subsequently received irinotecan (50mg/kg) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection; Groups III and IV, received oral dose of lutein (6mg/kg/day) and (24mg/kg/day), respectively by oral gavage for 25 successive days (lutein-treated); Groups V and VI (lutein+ irinotecan), received oral dose of lutein (6mg/kg/day) and (24mg/kg/day), respectively by oral gavage for 25 successive days, and subsequently received irinotecan (50mg/kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection.

Results: Orally-administered lutein with total cumulative dose of irinotecan (Groups V and VI), resulted in significant reduction (P<0.05) of serum aspartate aminotransferase and alanine aminotransferase, and significant reduction (P<0.05) of malondialdehyde; but, significant elevation (P<0.05) of serum total antioxidant capacity; and there was significant reduction in caspase 3 in liver tissues homogenates compared to the corresponding levels in the group of rats administered irinotecan (Group II).

Conclusion: Results of the current finding suggested that administration of lutein may be a useful compound that alleviated irinotecan induced toxicity to the liver.

Keywords: Lutein, Irinotecan, AST, ALT, Malondialdehyde (MDA), TAOC, Caspase 3 (Casp-3).

Introduction

The DNA topoisomerase enzymes are fundamental to cell function and abundantly found in all fields of life; and different topoisomerase enzymes precede a vast range of functions regarding to the maintenance of DNA topology within DNA transcription and replication; and are targets of a broad range of anti-microbial and cancer chemotherapeutic agents (1).

Irinotecan (CPT-11), a semisynthetic drug that was derived from the natural alkaloid plant camptothecin which was clinically introduced in the late of 1980s (2). Such drug is vastly used in the treatment of pancreatic, colorectal, and lung cancer; furthermore, irinotecan is a prodrug for the active metabolite SN-38, which is 100- to 1000-fold further cytotoxic more than irinotecan (3).

Authors reported that, the mechanism of the antitumor action of irinotecan is through the inhibition of DNA topoisomerase I that it can cause DNA damage
which consequently may lead to cell death in fast-proliferating natural cells such as intestinal basal cells and BM cells (4); the interaction of irinotecan with topoisomerase type I (Topo I)–DNA cellular complexes has cytotoxicity in S-phase-specific. Irinotecan also kill the non-S-phase cells at greater concentrations through mechanism related to DNA damage by transcriptionally induced and other mechanism of apoptosis (5); thus, treatment with irinotecan can be associated with gastrointestinal (GI) and hematologic toxicities; where, the main dose-limiting toxicities are sever delayed diarrhea and neutropenia, respectively (4).

Lutein is a carotenoid pigment; and it is a compound of xanthophyll group that found abundantly in kale, corn, animal fat, spinach, etc. (6). It cannot be synthesized by human body but can be provided only by diet or supplementation (7). Moreover, lutein can selectively be taken up into the macula of the eye where it thought to protect against the development of age-related macular degeneration (8). Also, It has been reported that lutein exhibited antioxidant, anti-genotoxic property, reduces inflammation and immunosuppression induced by ultraviolet radiation in mouse models and may have protective activity against macular degeneration due to age effect (6).

**Objectives**

The current study was designed to describe roles of oxidative stress and apoptosis as mechanisms of liver toxicity induced by irinotecan; and to explore the possible protective effects of high- and low- dose of lutein against irinotecan induced toxicity in the liver of rats.

**Materials and Methods**

**Reagents:** Rat’s kits Standards were obtained from SUNLONG BIOTECH CO., LTD, China.

**Drugs:** Irinotecan 100mg vials obtained from Fresenius Kabi, India and the pure powder of lutein was obtained from Xi’an Rongsheng Biotechnology Co., Ltd. China.

**Animals and experimental design**

Thirty six (36) adult Sprague-Dawley rats, each weighing 150-200gm were taken from The Animal House of the College of Pharmacy/ University of Baghdad, under controlled and conventional laboratory conditions; rats were housed in cages of stainless steel, of temperature (25°C), relative humidity and natural light/dark cycle. Standard laboratory rodent tap water and chow were supplied ad libitum, and the animals adapted for a one week period prior of the experiment. The animals were divided into six groups of six rats each as follows:

- **Group I (Control):** received single oral daily dose of dimethyl sulfoxide (DMSO) (4 ml/kg) for 25 consecutive days by oral gavage. This group served as control.
- **Group II (irinotecan-treated):** received single daily oral dose of dimethyl sulfoxide (DMSO) (4 ml/kg body weight/day) for 25 days, and subsequently received irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by IP injection.
- **Group III (6mg lutein/kg/day) (lutein-treated):** received oral dose of lutein (6mg/kg/day) daily by oral gavage for 25 consecutive days.
- **Group IV (24mg lutein/kg/day) (lutein-treated):** received oral dose of lutein (24mg/kg/day) daily by oral gavage for 25 consecutive days.
- **Group V (6mg lutein /kg/day + irinotecan):** received oral dose of lutein (6mg/kg/day) daily by oral gavage for 25 consecutive days, and subsequently received irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by IP injection.
- **Group VI (24mg lutein /kg/day+ irinotecan):** received oral dose of lutein (24mg/kg/day) daily by oral gavage for 25 consecutive days, and subsequently received irinotecan (50mg per kg body weight) on days: 5, 10, 15 (total dose=150 mg/kg) by intraperitoneal injection.

Twenty-four (24) hours after the end of treatment, all rats were euthanized by diethyl ether anesthesia and from each rat blood sample were withdrawn from the carotid artery at the neck and collected into the labeled centrifuging tubes and leaved to clot for 20 min at room temperature to obtain serum, which was separated by centrifugation at 3000 rpm for 20 min, and is utilized for the assessments of biochemical parameters [Aspartate
aminotransferase (AST), alanine aminotransferase (ALT), and total antioxidant capacity (T-AOC), levels.

Furthermore, rats’ liver were quickly excised, placed in chilled phosphate buffer solution (PBS) (pH 7.4) at 4°C, blotted with filter paper and weighed. For the preparation of 10% tissues homogenates, 9ml of PBS (pH 7.4) was added to 1gram of the liver, then the tissue was homogenized by tissue homogenizer that set at 3 for 1 minute at 4°C. All preparations were freshly prepared and kept frozen at (-180°C) unless worked immediately for the measurement of MDA and Casp-3 in liver tissue homogenates.

**Statistical Analysis**

Data was expressed as the values of mean±standard deviation (SD). Data were analyzed by utilizing computerized Statistical Package for the Social Sciences (SPSS) program. The statistical significance of the differences among various groups is determined by one-way analysis of variance (ANOVA). The statistically significant differences were considered when P value less than 0.05 (P<0.05).

**Results**

Irinotecan (Group II) cause significant (P<0.05) elevations in serum levels of AST (Fig. 1), and ALT (Fig. 2) each compared to the corresponding levels in control (Group I) rats; and, there were significant (P<0.05) reduction in serum levels of TAOC (Fig. 3); furthermore, there were significant (P<0.05) elevations in MDA contents (Fig. 4) in liver tissue homogenates compared to control (Group I) rats; similarly, there were significant (P<0.05) elevations in Casp-3 level in liver tissues homogenates of rats (Fig 5) compared to control (Group I) rats.

Groups III and IV rats that orally received lutein 6mg/kg and 24mg/kg, respectively each produced non-significant differences (P>0.05) in serum AST, ALT, and TAOC; also, there were non-significant differences (P>0.05) in MDA and Casp-3 in liver tissues homogenates with respect to corresponding levels in Group I rats (Figures 1-5, respectively).

Administration of lutein at a dose of 6mg/kg body weight, and 24mg/kg each in association with irinotecan (Groups V and VI respectively) significantly (P<0.05) reduced serum enzymes AST (Fig. 1), ALT (Fig. 2) and significantly (P<0.05) elevated serum TAOC (Fig. 3) levels with respect to Group II; Moreover, significant (P<0.05) reduction in MDA contents in liver tissues homogenates (Fig. 4) and significantly (P<0.05) reduced Casp-3 in liver tissues homogenates (Fig. 5) with respect to Group II.

By comparing various markers measured in groups of rats that received low versus high doses of lutein in association with irinotecan (Groups V and VI), there were significant (P<0.05) reduction in serum levels of AST (Fig 1), and ALT (Fig 2) in Group VI rats compared to the corresponding serum level in Group V rats. Furthermore, there were non-significant elevation (P<0.05) in serum TAOC (Fig. 3), non-significant (P<0.05) differences in MDA contents (Fig. 4) and in Casp-3 (Fig 5) in liver tissue homogenate of Group VI rats compared to the corresponding levels in Group V rats.

Additionally, by comparing various markers measured in groups of rats that received low and high dose of lutein in association with irinotecan (Groups V and VI) compared to control (Group I) rats, there were non-significant (P>0.05) differences in serum level of AST and ALT (Fig. 1 and 2) of Group VI compared to the corresponding levels in Group I rats; there were non-significant (P>0.05) differences in serum level of TAOC (Fig. 3); MDA contents (Fig. 4) and in Casp-3 (Fig. 5) in liver tissue homogenate of each of Group V and Group VI rats compared to the corresponding levels in Group I rats.
Fig. 1: Effects of lutein (6mg and 24mg) on irinotecan induced liver toxicity on serum aspartate aminotransferase (AST) in rats. Data are expressed as Mean±SD, n = 6. Values with non-identical small letters (a, b, and c) are significantly different (P< 0.05). Values with non-identical capital letters (A and B) are significantly different (P< 0.05).

Fig. 2: Effects of lutein (6mg and 24mg) on irinotecan induced liver toxicity on Serum Alanine Aminotransferase (ALT) levels in rats. Data are expressed as Mean±SD, n = 6.
Values with non-identical small letters (a, b and c) are significantly different (P< 0.05).

Values with non-identical capital letters (A and B) are significantly different (P< 0.05).

Fig. 3: Effects of lutein (6mg and 24mg) on irinotecan induced liver toxicity on serum Total antioxidant capacity (T-AOC) in rats.

Data are expressed as Mean±SD, n =6.

Values with non-identical small letters (a, and b) are significantly different (P< 0.05).

Values with non-identical capital letters (A) are significantly different (P< 0.05).

Fig. 4: Effects of lutein (6mg and 24mg) on irinotecan induced toxicity on MDA in liver tissues homogenates of rats.

Data are expressed as Mean±SD, n =6.
Discussion

Severe adverse effects of the clinical use of irinotecan including myelosuppression and diarrhea can limit the using of such drug in the aggressive therapy; where, myelosuppression, especially the neutropenia, was frequently-observed in patients received irinotecan chemotherapy (9). Moreover, authors mentioned that there was a link between irinotecan therapy and the development of steatohepatitis that pathologically characterized by inflammation, accumulation of fat (steatosis), hepatocytes ballooning, and fibrosis (4). Although exact mechanisms by which irinotecan caused steatohepatitis are not entirely understood, oxidative stress (OS) and mitochondrial dysfunction seems to play a main role (10).

The current study confirms that irinotecan caused liver toxicities, as was evidenced through the significant (P<0.05) elevations in MDA content (figure 4) in liver tissue homogenates; in addition, there were reduction in serum TAOC (figure 3) and elevation in serum AST, and ALT (figures 1 and 2, respectively).

The role of apoptosis as a mechanism provoked by irinotecan on its toxicity on liver was not previously described; but in this study, irinotecan caused significant elevation in Casp-3 level in liver tissue homogenate (Figure 5); and thus, the current study is considered the first that demonstrate the role of apoptosis in liver toxicity-induced by irinotecan. Thus, we did not have a chance to compare the results of this study with other reports concerning this respect.

Lutein was extensively studied for its anti-inflammatory and antioxidant neuro-protectant activity in various disease models like uveitis, diabetic retinopathy, ischemia/reperfusion injury, and light induced retinopathy (11). Furthermore, it has been reported that lutein exhibited anti-genotoxic property,
and may attenuate the immunosuppression induced by ultraviolet radiation in mouse models and may have protective activity against macular degeneration due to age effect; and it has activity in chemopreventive and can protect from macular degeneration due to age effect (6).

The current study showed that lutein (6mg and 24mg/kg/day) attenuates irinotecan -induced reduction in serum TAOC level and also attenuates irinotecan -induced elevation in serum AST, and ALT levels, and MDA contents and Casp-3 levels in liver tissues homogenates of rats (figures 1, 2, 3, 4 and 5).

**Conclusion**

Results of this study suggested that oxidative stress and apoptosis have roles in mechanisms of liver toxicity induced by irinotecan; besides, the utilized doses of lutein has protective effect on irinotecan-induced toxicity to the liver during the DNA topoisomerase enzymes inhibitor (irinotecan) chemotherapy.

**Acknowledgments:** The data of this article were abstracted from PhD thesis submitted to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. Authors thank University of Baghdad for supporting the project.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**

The Association between Severity of Dental Caries and Salivary Immunoglobulins in Asthmatic Adult Patients

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Abstract

The salivary samples were collected adequately from 80 subjects distributed equally (40 asthmatic subjects and 40 control subjects) of ages range between 20-60 years. This comparative survey was determining the caries index (DMFT), Streptococcus mutans, Streptococcus sobrinus, salivary IgA and IgE. So this study have shown highly significant difference P ≤ (0.01) found in effect of age on salivary IgA in control adult subjects and non-significant difference (P > 0.05) displayed in influence of age on salivary IgA. So this comparative investigation have demonstrated that non-significant difference (P > 0.05) found in influence of age on salivary IgE to both groups of study. However, the correlation between age and salivary IgE is a weak negative relationship.

Key words: Asthma, dental caries, salivary IgA, salivary IgE

Introduction

Asthma is a chronic disease causing airway inflammation in the lungs involving many cells and cellular elements. Worldwide, roughly 300 million people are affected by this disease irrespective of the age ¹. During the last few decades, disease has been elevated in some countries but has stabilized in other countries ²³. The asthma in period of adulthood is more frequent in females than in males ³. Today, the most active available therapy for asthma is anti-inflammatory inhalers (glucocorticosteroids) and, if necessary, the addition of ß2 agonists (bronchodilatory agents) ⁴.

It’s attributed to hyper-responsiveness of airways to triggers that impacts the sensitivity of the nerve endings in the air passages of lungs and so they become easily excited, for example exercise as well as allergens, which causes recurrent symptoms e.g. dyspnea (shortness of breath), coughing, chest tightness and wheezing ⁵. This disease can be considered as the most common in childhood and is also a major problem of public health in adult populations. In an attack, the lining of the pathways swell resulting the narrowing of the airways and decreasing the passage of air in and out of the lungs ⁶. The basic mechanism of asthma is an inflammatory process in the airways. The highest prevalences of asthma were recorded in Peru, Australia, New Zealand and the lowest recorded prevalence was documented in Albania and Russia ⁷.

The asthmatic patients have many problems in oral health. One of these problems is dental caries (DMFT index) which characterized by an irreversible contagious disease of multi-factorial etiology resulting demineralization of inorganic elements and destruction of an organic part of tooth structure ⁸. Several surveys have been shown an elevated incidence of dental caries in asthmatic patients ⁹. The increased incidence of caries is attributed to: low salivary PH (reduced buffering capacity), low salivary flow rate, raised consumption of carbonating drinks and sweets and magnified desiccating effect due to mouth breathing. Mutans streptococci (MS) is dominant cariogenic bacteria considered to be high in asthmatic subjects as compared to non-asthmatic subjects due to decreased salivary flow rate ¹⁰.

Materials and Method

Sugar Bacitracin-20M (Sugar bacitracin -20 modified)agar (which is obtained from Bulgaria), Human IgE ELISA kit(that we got from Al-shkairate
establishment for medical supply, Jordan) and Salivary IgA ELISA kit (which is got from LDN, Germany)

**Method**

This comparative study involved the salivary samples of 40 control subjects and 40 asthmatic patients of ages between 20-60 years according to the birthday. Collecting the saliva was made in early morning between 8am to 9 am. Prior to collecting the saliva, subjects were advised not to drink or eat for 3 hours before this step. Around 1 to 3 ml of total unstimulated saliva was assembled easily by drooling into sterilized cups that possess graduations, with the forward tilted head or by permitting the accumulation of saliva in the mouth and then expectorate into a cup (to avoid any possible contamination), while the determination of salivary flow rate (ml/min) must be done by using the graduated cup as (volume of saliva sample collected is divided on time needed for collecting the saliva) ml/min. Then taking 0.5 ml by micropipette for serial dilution tube, and the resulting saliva should be centrifuged for 3000 rpm for 10 min., and the clear supernatant saliva was collected and stored in a freezer at -20°C. The use of Vortex mixer for two minutes so that salivary samples be homogenous. Tenfold steps of serial dilution were prepared, 0.1 ml was withdrawn from each dilution (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵) and spreading by use of sterile microbiological spreaders on the petri dish plates of SB-20M agar.

These plates were incubated anaerobically by using a gas pack provided in an anaerobic jar for 24 hours at 37°C followed by aerobic incubation for 24 hours at 37°C. such experiment was accomplished in duplicate.

**Results and Discussion**

The caries index (DMFT), microbial counts, salivary IgA and salivary IgE in two study groups are demonstrated in table 1 and table 2. The results displayed the mean rank of DMFT in control group is higher in males as compared with females. Also the mean rank of microbial counts represented with Streptococcus mutans, Streptococcus sobrinus and total streptococci counts in males is higher than that of females. As related to the salivary IgA and salivary IgE, there is non-significant differences (P > 0.05) between the gender and both salivary immunoglobulins (IgE and IgA) as shown in table 1.

As for asthma group, table 2 demonstrates non-significant differences (P > 0.05) between influence of gender on DMFT index, microbial counts and salivary immunoglobulins represented with salivary IgE and IgA.

**Table 1: Descriptive statistics and gender difference for the variables in control group**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (N=28)</th>
<th>Females (N=12)</th>
<th>Gender difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Age</td>
<td>41</td>
<td>20</td>
<td>59</td>
</tr>
<tr>
<td>DMFT</td>
<td>10</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>S. mutans count</td>
<td>6.15</td>
<td>1.3</td>
<td>16.4</td>
</tr>
<tr>
<td>S. sobrinus count</td>
<td>1.35</td>
<td>0.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Total Streptococci count</td>
<td>7.4</td>
<td>1.7</td>
<td>21.5</td>
</tr>
<tr>
<td>IgA</td>
<td>179</td>
<td>73</td>
<td>641</td>
</tr>
<tr>
<td>IgE</td>
<td>12.6</td>
<td>2.1</td>
<td>31.6</td>
</tr>
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</table>
Table 2: Descriptive statistics and gender difference for the variables in Asthmatic group.

<table>
<thead>
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<th>Variables</th>
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<th>Gender difference</th>
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<tr>
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<td>Males (N=24)</td>
<td>Females (N=16)</td>
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<tr>
<td></td>
<td>Median</td>
<td>Min.</td>
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<tr>
<td>DMFT</td>
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<td>S. mutans count</td>
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<td>2.7</td>
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<tr>
<td>S. sobrinus count</td>
<td>2.05</td>
<td>0.6</td>
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<tr>
<td>Total Streptococci count</td>
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<td>IgA</td>
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<td>IgE</td>
<td>14.4</td>
<td>1.6</td>
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Table 3: Relation between the DMFT and all variables in both groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Age</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
</tr>
<tr>
<td>S. mutans count</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
</tr>
<tr>
<td>S. sobrinus count</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
</tr>
<tr>
<td>Total Streptococci count</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
</tr>
<tr>
<td>IgA</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
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<td>r</td>
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<td>p-value</td>
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Table 4: Relation between the IgA and all variables in both groups

<table>
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<td>-0.267</td>
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<tr>
<td></td>
<td>p-value</td>
<td>0.000</td>
<td>0.096</td>
</tr>
<tr>
<td>S. mutans count</td>
<td>r</td>
<td>-0.165</td>
<td>-0.103</td>
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<tr>
<td></td>
<td>p-value</td>
<td>0.308</td>
<td>0.526</td>
</tr>
<tr>
<td>S. sobrinus count</td>
<td>r</td>
<td>-0.013</td>
<td>-0.087</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.937</td>
<td>0.594</td>
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<tr>
<td>Total Streptococci count</td>
<td>r</td>
<td>-0.145</td>
<td>-0.095</td>
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<tr>
<td></td>
<td>p-value</td>
<td>0.372</td>
<td>0.558</td>
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<td>IgE</td>
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<td>-0.275</td>
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<td>p-value</td>
<td>0.086</td>
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Table 5: Relation between the IgE and all variables in both groups

<table>
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<th>Asthma</th>
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<tr>
<td>Age</td>
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<td>-0.087</td>
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<tr>
<td></td>
<td>p-value</td>
<td>0.240</td>
<td>0.592</td>
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<tr>
<td>S. mutans count</td>
<td>r</td>
<td>0.007</td>
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<tr>
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<td>p-value</td>
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<td>0.637</td>
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<td>S. sobrinus count</td>
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<td>0.110</td>
<td>-0.065</td>
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<td></td>
<td>p-value</td>
<td>0.500</td>
<td>0.689</td>
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<tr>
<td>Total Streptococci count</td>
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<td>0.035</td>
<td>0.059</td>
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<tr>
<td></td>
<td>p-value</td>
<td>0.830</td>
<td>0.717</td>
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In this comparative study, results display there is a weak positive relationship of DMFT with age in both groups of study with non-significant differences (P > 0.05) between DMFT and age in control group and asthma group. Also results indicate highly significant (P ≤ 0.01) difference between DMFT and S. mutans count in both groups. Also there is highly significant difference (P ≤ 0.01) between DMFT and S.sobrinus in both groups. Subsequently, the difference is highly significant (P ≤ 0.01) between DMFT and total Streptococci count in both groups of study. Low salivary PH (reduced buffering capacity), low salivary flow rate and other parameters
are increasing severity of caries and subsequently elevation of MS levels in asthmatic patients. However, there is non-significant differences (P > 0.05) between DMFT index and both salivary immunoglobulins (IgA and IgE) in control group and asthma group. At the same time, the correlation is a weak negative relationship between DMFT and salivary IgA in both groups, but the correlation between DMFT and salivary IgE is a weak positive relationship in control group and weak negative relationship in asthma group. Relative there is an increase in mean values of IgE in asthma group as compared with control group. Increased Th2 levels in the airways liberate specific cytokines which stimulate inflammation of eosinophils and generation of IgE(immunoglobulin E) by mast cells.

In regards to salivary IgA, results illustrates the mean value in control group is higher than that of asthma group and this is may be due to taking β2 agonists that aggravate the severity of caries by decrease the salivary flow rate and reduce levels of salivary IgA in asthmatic patients. So the correlation this salivary parameter with age in control group is a strong positive relationship and the difference is highly significant (P ≤ 0.01), but in asthma group, there is non-significant difference (P > 0.05) between salivary IgA and age with taking in consideration that there is a weak negative relationship. So the results demonstrated that there is a weak negative relationship between salivary IgA and S. mutans count as well as the correlation between salivary IgA and S. sobrinus count is also a weak negative relationship.

As for the correlation between both salivary immunoglobulins (IgA and IgE) is a weak negative relationship whether in control group or till in asthma group. So the statistical difference between salivary IgA and IgE is non-significant (P > 0.05).

Results displayed that in both groups of study there is a weak negative relationship between IgE and age as well as non-significant statistical difference (P > 0.05). So the correlation in both groups is a weak positive relationship between IgE and S. mutans count with non-significant statistical difference (P > 0.05), but the correlation is a weak positive relationship between IgE and S. sobrinus count in control group and a weak negative relationship between them in asthma group. So statistically there is non-significant difference (P > 0.05) between IgE and S. sobrinus in control group and asthma group.

Conclusion

As related to the low salivary flow rate and low PH in asthmatic adult patients, the levels of dental caries index (DMFT), S. mutans and S. sobrinus are higher in asthma group than that in control group.

So the mean value of IgA is higher in control subjects than that in asthmatic adult patients. So the mean value of IgE in asthmatic subjects is higher than that in control subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

5- Global Initiative for Asthma (GINA). 2009 ,Global strategy for asthma management and prevention.


Phenotypic and Molecular Characteristics of Biofilm and other Virulence Genes in *E. coli* and *K. pneumoniae* Isolates from Healthy Dairy Cow, Human and Environmental Sources

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¹Biologist, ²Prof. Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq

Abstract

*Escherichia coli* and *Klebsiella pneumoniae* for ones role in opportunistic infections and pathogenic methods in veterinary and human medicine, including the formation of biofilms. 33 *E. coli* and 8 *K. pneumoniae* isolates of these micro-organisms isolated from cow’s milk, stools and utensils. Identification were done by biochemical reaction and confirmed by Polymerase Chain (PCR). These isolates exhibit biofilms formation, as strong, moderate, weak capacities. The expression of the *fimA* gene. The percentage (84.8%) 28/33 of *E. coli* was able to produce biofilm while (100%) 8/8 in *K. pneumoniae*. The virulence factors of all bacterial isolates were also studied to determine *hylA* and *bla-CTX-M* genes in *E. coli* and, *magA* and *bla-CTX-M* genes in *K. pneumoniae*. were results presence extended spectrum β- lactamases (ESBLs). *bla-CTX-M* gene in *E. coli* and *K. pneumoniae* (78.7%, 87.5%) It also emerged that not all isolates carry other virulence genes for this study.

**Key words :** biofilm, virulence genes, *E. coli*, *K. pneumoniae*

Introduction

Microbial adhesion onto, surfaces and therefore the biofilm formation are considered serious, regarding their economical and public health consequences in many sectors for humans. The presence of pathogenic microorganisms on food sector facilities represents a severe potential health risk to consumers. Contaminated food contact with surfaces promotes contamination of food products which leads to foodborne disease³. With this pathogen contamination, of bulk tank milk by Shiga toxin-producing *E. coli*. therefore, milk is at risk for contamination by any pathogen that is present in feces or farm environment². Grass-fed cattle are the main reservoir of such *E. coli* strains. Some *E. coli* strains can produce toxins that induce serious human infections. cross-contamination of milk can occur in utensils contamination and during subsequent handling and preparation. Their feces might contaminate and thus act like a microbial carrier which might end up contaminating other foods such as milk, outbreaks due to *E. coli* 0157: H7 and *klebsiella* spp. have been associated primarily¹. The ability to adhere to different materials and formed biofilms have been an important factor associated with *E. coli* and *klebsiella* spp. virulence additionally, resistance to antimicrobials in biofilm-forming isolates contributes to bacterial persistence which may lead to chronic infections and treatment problem⁴. *Escherichia coli* and *Klebsiella pneumoniae* are not only constituents of the commensal gut flora but also common opportunistic pathogens in intestinal infection and implicated in the urinary tract and bloodstream infections. They frequently harbor ESBL- encoding genes. Broad-spectrum beta-lactamase production is associated with increased infections and mortality⁴ virulence *E. Coli* capsule with polysaccharide. More than 80 different types of capsules, *E. Coli* capsules are categorized into four major classes according to the genetic organization of the capsule gene cluster and its biosynthesis and assembly mechanism⁵. To *K. Pneumoniae* usually expresses on its surface a smooth lipopolysaccharide (LPS with O antigen) and capsule polysaccharide (K antigen) both leading to the pathogenesis of microorganism diseases caused by this species⁶,⁷
Material and Methods

Samples (190) were collected from different locations (60 cows milk, 60 stool human and 70 utensils samples). The collected samples were immediately, delivered to the laboratory in a cool box and tested within 24 h.

All E.coli and Klebsiella pneumoniae isolates grow readily on MacConkey agar and colonies typically appear large and pink, with pink pigment usually, diffusing into the surrounding agar indicating, fermentation of lactose. Klebsiella pneumoniae show the colonies mucoid on MacConkey agar. However E. coli colonies have a characteristic green metallic sheen on EMB agar. The API-20 E kit (bioMérieux – France), was used for confirmation the identification of isolates.

The method of DNA, extraction was done by using (Geneaid extraction Kit). The concentration of DNA, was estimated by using the nanodrop system 8.

Biofilm formation was assayed phenotypically by the ability of cells to adhere to the wells of 96-well microtiter plate 9. Two hundred microliters of this bacterial culture were used to inoculate pre-sterilized 96-well polystyrene microtiter plates and later incubated for 48 hrs. at 37°C, all wells were washed with sterile physiological saline for the elimination of unattached cells. Afterward, 200 µl of 1% crystal violet was added to each well. After 15 min. at room temperature, each well was washed with 200 µl sterile physiological saline. This process was repeated three times. The crystal violet bound to the biofilm was extracted later with 200µl of ethyl alcohol, and then absorbance was determined at 540 nm in an ELISA reader 10.

Specific primer sequences in PCR assays, and the predicted size of the amplified products, for the different pathogenic gene, coding regions were employed (Table 1). The reaction volume for magA, hylA, fimA and bla-CTX-M genes was 20 µl, while the amplification program was 3 at 95 C for denaturation, 30 cycles for denaturation 95 C (45 Sec.) ,annealing 60 C (45 Sec.) and extension 72 C for 50 Sec. with final extension 72 for 10 Min.

Table 1 Sequences and predicted lengths of PCR amplification, products of the oligonucleotide primers used

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5-3)</th>
<th>Product</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>fimA</td>
<td>F 5 AGTTAGGACAGGTTCTGACCGCAT-3 R 5- AAATAACGCGCCTGGAAACGAATG-3</td>
<td>316bp</td>
<td>11</td>
</tr>
<tr>
<td>bla-CTX-M</td>
<td>F5”TCTTCCAGAATAAGGAATCC”3 CCGTTGCCCTATTACAC”3 R5”</td>
<td>909bp</td>
<td>12</td>
</tr>
<tr>
<td>magA</td>
<td>TAGGTCAGGCGACTGTTG “3 F”5 GCTCGTTTGCAATATGCCG “3 R5”</td>
<td>312bp</td>
<td>13</td>
</tr>
<tr>
<td>hylA gene E. coli</td>
<td>“3 F-5” TGAAGTGTCCAGGAGACCTG “3 R-5” ATGGAGAATGCGTTCCTCAAC</td>
<td>156bp</td>
<td>27</td>
</tr>
</tbody>
</table>
Results

The raw milk, stool and utensils samples displayed the presence of *E.coli* and *klebsiella pneumonia* contamination. The raw milk samples were polluted with the fecal coliform according to the results of fermentative growth in MacConky. Out of 190 samples (milk, stool and utensils) were collected from different sources, 33 samples (17.36%) were contaminated by *E.coli* isolates. While eight samples (4.21%) *Klebsiella pneumoniae* were confirmed. All *E. coli* 33 isolates were produce biofilm (100%). Out of 33 isolates 14 (42.42%), were strong biofilm producer. The high percentage of isolates with strong biofilm produce were in stool isolates 10/19 (52.63%). The moderate biofilm produce isolates were 8/33 (24.24%), While the weak biofilm produce isolates were 11/33 (33.33%). Also all *K. pneumoniae* isolates were able to produce biofilm (100%). Out of a 8 tested *Klebsiella pneumoniae* isolates by using pre-sterilized 96-well polystyrene microtiter plates (Fig.1) 5/8 (62.5%) of isolates were moderate biofilm producers and the remaining isolates 3/8 (37.5%) were weak biofilm, producer.

![Figure 1: Pre-sterilized 96-well polystyrene microtiter plates biofilm production.](image)

From 33 *E.coli* isolates, 28 (84.8%) were carried the genes responsible on biofilm formation *E. coli* and 8 (100%) *k. pneumoniae* were had fimA gene responsible for biofilm formation (Table 2, Fig 2). The detection of hylA gene in *E.coli* and magA gene in *k. pneumoniae* while bla-CTX-M in both. The results showed that hylA gene and magA gene were not found an all *E. coli* and *K. pneumoniae* isolates respectively, while *CTX-M* gene was found in 26/33 (78.7%) of *E. coli* isolates, while in *K. pneumoniae* isolates was found in percentage 7/8 (87.5%), table 3, Fig 3.
Table 2: Distribution of fimA gene in E. coli and K. pneumoniae isolates

<table>
<thead>
<tr>
<th>Sample</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>fimA NO./ %</td>
</tr>
<tr>
<td>Stool</td>
<td>19</td>
<td>16/84.2</td>
</tr>
<tr>
<td>Milk</td>
<td>11</td>
<td>9/81.8</td>
</tr>
<tr>
<td>Utensils</td>
<td>3</td>
<td>3/100</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>28/84.8</td>
</tr>
</tbody>
</table>

Figure (2): Gel electrophoresis, of amplified fimA gene, using conventional PCR. Agarose, 2%, and TBE(1X), at (75 V/cm for 90 min., stained with RedSafe™ and visualized on a UV transilluminator. Marker (M): (100-3000bp), Lanes:(1-8) Milk Samples

Table 3: Distribution of bla-CTX-M gene in E. coli and K. pneumoniae isolates

<table>
<thead>
<tr>
<th>Sample</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>bla-CTX-M NO./ %</td>
</tr>
<tr>
<td>Stool</td>
<td>19</td>
<td>15/78.9</td>
</tr>
<tr>
<td>Milk</td>
<td>11</td>
<td>9/81.8</td>
</tr>
<tr>
<td>Utensils</td>
<td>3</td>
<td>2/66.6</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>26/78.7</td>
</tr>
</tbody>
</table>
Figure 2: Gel electrophoresis of amplified Bla-ctx-m gene, using conventional PCR. Agarose, 2%, and TBE(1X), at (75 V/cm for 90 min.), stained with RedSafe™ and visualized on a UV transilluminator. Marker (M): (100-3000bp), Lanes: (1-8) stool samples.

Discussion

In this study, a high percentage of agreement, was observed for the phenotypes of isolates, determined by microtiter plate applicability, reliability and high reproducibility of the microtiter plate were previously verified for bacterial biofilm\textsuperscript{15}. The isolated \textit{E. coli} and \textit{K. pneumoniae} were evaluated for biofilm formation, by using phenotypic microtiter plate screening as well as molecular detection of genes. Microtiter plate (MTP) showed that 33 isolates of \textit{E. coli} were biofilm producers, 42.2\% of isolates were strong, 24.2\% were moderate and 33.3\% were weak biofilm producers. This study consistent with results of previous study by\textsuperscript{32}. While eight isolates of \textit{K. pneumoniae}, were able to form biofilm percent 62.5\% moderate and 37.5 weak. These data are in accordance with those reported by\textsuperscript{17}.

\textit{E.coli} tends to be the most important cause of infections associated with mastitis. However, more precise investigation of individual farms revealed a farm-specific infection pattern where a single Gram-negative bacterial species, perhaps there is subclinical mastitis or intra mammary infection\textsuperscript{18}.

The \textit{fimA} gene is responsible for the formation of biofilms. Type 1 fimbriae are encoded by \textit{fimA} gene, which can facilitate bacterial adhesion and biofilm formation\textsuperscript{26}. This study was detected production of \textit{fimA} gene in 84.8\% of \textit{Escherichia coli} isolated from stool, milk and utensils where as found in Table (4). Result in this study is an agreement with a result of a study done by\textsuperscript{20}. Another study had been noticed less percentage in study done by\textsuperscript{21}. Who founded 76\%. This study was revealed that all \textit{K. pneumoniae} isolates wich was all have been positive for the presence of \textit{fimA} gene in 8/8100\%. This result agreement with result of\textsuperscript{21}. who showed the presence of \textit{fimA} and \textit{fimH} genes in 100\%.

The investigation and detection of \textit{blaCTX-M} gene in \textit{E.coli} isolates revealed in this study the presence of this gene in percentage 78.7\% (26/33) this result near from study done by\textsuperscript{22}.

In \textit{K. pneumoniae} the result presence of a \textit{blaCTX-M} gene was found in percentage 80\%. But this result was less than the result by\textsuperscript{25} in China who mentioned the percentage was 87.62\%. But it was higher than the result reported by\textsuperscript{24}, who reported 30\%, the other hand the \textit{hylA} gene was not detected in all \textit{E.coli} that similar to studies findings were obtained by\textsuperscript{20}. While disagree with the result reported by\textsuperscript{20}. while was \textit{magA} gene in all were negative for \textit{K. pneumoniae} isolates this result agreement with result reported \textsuperscript{27} but was differ from the result reported in AL-diwaniyah city, Iraq who recorded \textit{magA} gene in percentage 92\% by\textsuperscript{22}. Variations in the geographical distribution of Serotypes of \textit{K. pneumoniae}, were identified by\textsuperscript{28}. 


The presence of multiple genotypes and a high degree of genetic variation in *K. pneumoniae* isolates has also been confirmed by\(^2\). A study reported by\(^3\) that there are plasmid-carried genes that can express capsular polysaccharide synthesis (cps) genes.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** The author declare there is no conflict of Interest.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq. All experimental protocols were approved under the College. while experiments were carried out in accordance with approved guidelines.

**References**


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<tr>
<td>24.</td>
<td>Idbeis HI, Khudor MH. detection of intracellular adhesion gene (icaa and icad) and biofilm formation <em>staphylococcus aureus</em> isolates from mastitis milk milk cow.kufa jornal of vet. Med. Sciences 2019. Vol.10, No.1,</td>
</tr>
</tbody>
</table>
The Effect of Exercises by Pressing -Style to Developing A Man-to-Man Defense for Young Football Players

Qasim Muhammad Ali Hazim¹, Ahmed Abdulameer Hamzah²

¹Ph.D. Student, Faculty of Physical Education and Sports Sciences/ University of Babylon, Iraq, ²Prof. Faculty of Physical Education and Sports Sciences/ University of Babylon, Iraq

Abstract

The importance of the research is that it is a research attempt that is concerned with consolidating the idea of a pressing play style in the reality of Iraqi clubs and teams training through this defensive method to achieve the desired goal, as well as experimenting with the use of defensive exercises characterized by rapid application and high accuracy together, and to know the extent of the impact of exercises in a pressing style of play in developing defense man to man for young football players in a step aimed at strengthening the sports field with practical experiments and service to researchers in the sports field. The research objectives to prepare pressing play style exercises to develop defense, man to man, for young football players. To identify the effect of exercises in a pressing play style in developing defense, man to man for young football players. The researchers used the experimental method to design the two equivalent groups (experimental and control) with a pre and post-test that is consistent with the nature of the research problem. The research sample was chosen by a simple random method (lottery), where the experimental group was represented by the Al Shuohda youth sports club, whose number was (22) players, and the control group was represented in the Nahrain youth sports club, whose number is (21), (10) players were chosen from each group, experimental and control group by the deliberate method, and the research variables included the defense man to man (Close defense - flexible defense - cutting and acquisition - cutting and farthest). The researchers used statistical methods (mean, standard deviation, coefficient of torsion, Levin’s coefficient, t-test for correlated and non-correlated samples), and after presenting, analyzing and discussing the results, the researchers reached the following conclusions: pressing play style exercises developed man-to-man defense with two types of close defense and flexible defense for the experimental group under study. pressing play developed cutting, possession, cutting and farthest.

Keywords: exercises, pressing-style, defense, young, football players

Introduction

The offensive and defensive side in football is considered as complementary to the other, as the attack enables the team to achieve goals and win ¹. As for the defense, its mission is to keep dangerous attacks away from the goal and cover the nearby and most important areas that may cause problems for the team if they are not properly monitored. Continuous pressure on the players of the opposing team, each according to his position and the type of defense assigned to him, as each player has duties on the field of defense and offensive. From the above, we see the necessity of using modern methods and techniques in the process of sports training, as well as playing with modern methods that can serve the method that the coach deems appropriate during the competition, especially with the diversity of methods used by competitors, and from here lies the importance of research as it is a research attempt concerned with establishing the idea of pressing play ² style in the reality of Iraqi clubs and national team training through this defensive method to achieve the desired goal, as well as experimenting with the use of defensive exercises characterized by rapid application and high accuracy together, and knowing the impact of exercises in the pressing play style to developing defense man to man for young football players in a step aimed at strengthening the sports field practical experiments and service for

DOI Number: 10.37506/ijfmt.v15i1.13770
researchers in the field of sports. The football game is in constant development and this development came as a result of modern methods based on scientific foundations through studies, research, analysis and correct planning and did not come in vain or random. These methods have significantly and remarkably developed the performance of the teams, some of which focus on attack, some of which focus on defense, including what focuses on attack and defense, according to what each team has, and among the methods that have emerged in recent times is the compressive style of play, which made a clear shift for the teams that used it in terms of results in the local league or external participations, and from the teams that used this method is the English club Liverpool, led by the German coach (Jürgen Klopp), who was known for this style in the 2015 season in the English premier league.

And through the experience of the two researchers, being former players in the Iraqi premier league and holders of the (C and B) Asian football tournament, and through their follow-up to the Iraqi premier league and the Iraqi teams, the compressive style of play was not used as a style famous for in local or foreign competitions, but rather is used poorly during competitions and matches as well. The researcher noticed weakness in the performance of defensive duties, including man-to-man defense, who is characterized by close control of competitors, especially in areas close to the goal and scoring areas, which are the areas from which most goals are scored, as well as a weakness in the process of retrieving the ball through cutting, possession or cutting and farthest.

**Methodology**

The researchers used the experimental method to design the two equivalent groups (experimental and control) with pre and post criteria that are compatible with the nature of the research problem, and to achieve the desired goals.

The research community included Wasit governorate clubs for youth participating in the first-class Iraqi football league of (10) clubs, and the research sample was chosen by a simple random method (lottery), where the experimental group was represented by the Al Shuohda sports club for youth of (22) players, and the control group was represented by the Nahain sports club For youth, their number is (21), and (10) players have been identified from each experimental and control group by deliberate method. The researchers conducted homogenization of the sample members in terms of length, mass, and training age.

<p>| Table (1) Show the homogeneity coefficients of the research sample in length, training age, and mass. |
|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>N</th>
<th>Variables</th>
<th>Measurement units</th>
<th>experimental mean</th>
<th>Std. Deviation</th>
<th>control mean</th>
<th>Std. Deviation</th>
<th>levene</th>
<th>Skew ness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Length</td>
<td>cm</td>
<td>171.00</td>
<td>2.943</td>
<td>169.50</td>
<td>3.000</td>
<td>0.1</td>
<td>0.512</td>
</tr>
<tr>
<td>2</td>
<td>Mass</td>
<td>Kg</td>
<td>69.500</td>
<td>6.650</td>
<td>68.50</td>
<td>4.654</td>
<td>0.41</td>
<td>0.811</td>
</tr>
<tr>
<td>3</td>
<td>Training age</td>
<td>Month</td>
<td>53.88</td>
<td>4.272</td>
<td>54.22</td>
<td>6.233</td>
<td>0.511</td>
<td>0.624</td>
</tr>
</tbody>
</table>

The table shows the homogeneity of the variables, as the values are confined to (± 1) in the torsion coefficient, and the coefficient of levene was greater than the level of significance (0.05).

**Field research procedures:**

To measure man-to-man defense, the two researchers made a tournament for four clubs (Al-Shuhada, Al-Nahrain,
Al-Kut and Wasit), including the club that represented the experimental group and the club that represented the control group, where the researcher made a draw in a simple random way to choose the two clubs in the competition prepared by the researchers, in order to avoid bias for any of the research community clubs and to ensure that the experimental research variables are preserved. The first half was filmed only in order to involve the research sample for the two clubs and not to switch them during the first half of the competition, these matches were presented to the expert (Abdul Amir Naji) former captain of Al-Zawra Club and an analyst for Al-Iraqiya sport channel, who analyzed the matches through the (Dart Fish) analytical program through which defense data are given a man to a man and this procedure was done in the pre and post-tests of the subject of study without any change in circumstances.

Pre-tests:

The researchers held a four-way tournament that started on Wednesday 8/1/2020 at three o’clock in the afternoon at the Al-Shuhada club stadium, which represents the experimental group, and the Al-Nahrain club stadium, which represents the control group, with two matches every day and a two-day break between one match and another, and the championship lasted for a week until 14/1 / 2020.

Equivalence was performed on the two research groups by conducting tests for the research variables, as the researchers conducted a parity process for the two groups (the experimental group that applied the pressing play style and the control group that applied the coach’s style), and to find parity between the two groups, the researcher used the variables to be developed by conducting the tests and measurements used In the search represented in table (2).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measurement units</th>
<th>experimental mean</th>
<th>Std. Deviation</th>
<th>control mean</th>
<th>Std. Deviation</th>
<th>T test</th>
<th>Level sig</th>
<th>Type of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close defense</td>
<td>Number</td>
<td>8.333</td>
<td>2.214</td>
<td>8</td>
<td>1.732</td>
<td>0.158</td>
<td>0.882</td>
<td>Non sig</td>
</tr>
<tr>
<td>Flexible defense</td>
<td>Number</td>
<td>4.666</td>
<td>0.577</td>
<td>4.333</td>
<td>2.081</td>
<td>0.267</td>
<td>0.802</td>
<td>Non sig</td>
</tr>
<tr>
<td>Cutting and farthest</td>
<td>Number</td>
<td>34.66</td>
<td>4.041</td>
<td>32</td>
<td>3.605</td>
<td>0.853</td>
<td>0.442</td>
<td>Non sig</td>
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<tr>
<td>Cutting and acquisition</td>
<td>Number</td>
<td>20.666</td>
<td>1.577</td>
<td>19.333</td>
<td>3.785</td>
<td>0.603</td>
<td>0.579</td>
<td>Non sig</td>
</tr>
</tbody>
</table>

Significant at a significance level (0.05) if the confidence level is ≤ or = (0.05)

The main experience:

The researchers, after reviewing the scientific sources and previous studies, prepared exercises by pressing play style, which aim to develop man-to-man defense, and these exercises are in line with the nature and conditions of football matches, and they are distributed in the training units appropriately taking into account the components of pregnancy training (intensity, size and rest) during three days a week (Sunday, Tuesday and Thursday) in order for these exercises to be able to achieve development in man-to-man defense in football. These exercises will be applied during the start of the main experiment.
Post-tests:

After completing the main experiment, post-tests were conducted for the research sample, a quadruple championship was held for the same clubs participating in the pre-test, namely (Al-Shuhada, Al-Nahrain, Al-Kut and Wasit), where the championship was held on Tuesday 17/3/2020 at three o’clock afternoon, with two matches a day, a match at the Al-Shuhada club stadium, a match at Al-Nahrain club, and a two-day break between one match and another, and the tournament continued until Monday 23/3/2020, as the tournament lasted for seven days.

Statistical methods used:

The researchers used the statistical bag (spss) to analyze the research results, including:

- Mean.
- STD.EV.
- Skew ness.
- leven coefficients.

Presentation, analysis and discussion of results:

Presentation of the results of defense values one man to man and the percentage of possession for the control and experimental group and their analysis:

Table (3) shows the mean and standard deviations for the pre and post-tests, the calculated (t) value, the level of significance and the significance of the differences in the defense values of man to man for the control group.

<table>
<thead>
<tr>
<th>N</th>
<th>Variables</th>
<th>units</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>T test</th>
<th>F</th>
<th>A. F</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>mean</td>
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<td>mean</td>
<td>Std. Deviation</td>
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<td>Number</td>
<td>8</td>
<td>1.732</td>
<td>9.333</td>
<td>1.527</td>
<td>0.718</td>
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<td>3.214</td>
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<tr>
<td>2</td>
<td>Flexible defense</td>
<td>Number</td>
<td>4.333</td>
<td>2.081</td>
<td>7.330</td>
<td>1.577</td>
<td>2.598</td>
<td>3</td>
<td>2</td>
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<tr>
<td>3</td>
<td>cutting and farthest</td>
<td>Number</td>
<td>32</td>
<td>3.605</td>
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<td>4.785</td>
<td>1.097</td>
<td>4.667</td>
<td>7.371</td>
</tr>
<tr>
<td>4</td>
<td>cutting and acquisition</td>
<td>Number</td>
<td>17.3</td>
<td>3.785</td>
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<td>0.866</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
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<td>Close defense</td>
<td>Number</td>
<td>8.33</td>
<td>2.214</td>
<td>16.33</td>
<td>1.528</td>
<td>6.928</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Flexible defense</td>
<td>Number</td>
<td>4.66</td>
<td>0.577</td>
<td>11.66</td>
<td>1.562</td>
<td>12.124</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>cutting and farthest</td>
<td>Number</td>
<td>34.66</td>
<td>4.041</td>
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<td>5.250</td>
<td>13.66</td>
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</tr>
<tr>
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<td>cutting and acquisition</td>
<td>Number</td>
<td>20.66</td>
<td>1.577</td>
<td>26.66</td>
<td>1.527</td>
<td>10.392</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

Significant at a significance level (0.05) if the confidence level is ≤ or = (0.05)
Presentation, analysis and discussion of the results of the defense values, man-to-man, for the post-test of the control and experimental groups:

Table (4) shows the mean, standard deviations of the post-test, the calculated (t) value, the level of significance, and the significance of the differences in the man-to-man defense values between the control and experimental groups:

<table>
<thead>
<tr>
<th>N</th>
<th>Variable</th>
<th>Unit</th>
<th>experimental mean</th>
<th>Std. Deviation</th>
<th>control mean</th>
<th>Std. Deviation</th>
<th>T test</th>
<th>Level sig</th>
<th>Type sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Close defense</td>
<td>Number</td>
<td>16.33</td>
<td>1.528</td>
<td>9.333</td>
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<td>5.612</td>
<td>0.005</td>
<td>Sig</td>
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<tr>
<td>2</td>
<td>Flexible defense</td>
<td>Number</td>
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<td>1.562</td>
<td>7.330</td>
<td>1.577</td>
<td>9.192</td>
<td>0.001</td>
<td>Sig</td>
</tr>
<tr>
<td>3</td>
<td>cutting and farthest</td>
<td>Number</td>
<td>48.33</td>
<td>2.876</td>
<td>36.66</td>
<td>4.785</td>
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<td>Sig</td>
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<td>4</td>
<td>cutting and acquisition</td>
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<td>1.527</td>
<td>17.3</td>
<td>1.528</td>
<td>7.483</td>
<td>0.002</td>
<td>Sig</td>
</tr>
</tbody>
</table>

Significant at a significance level (0.05) if the confidence level is ≤ or = (0.05)

Discussion of the post-test results of the experimental and control group in man-to-man defense.

The researchers attribute the reason for the development of the experimental group to the use of the stress playing style that had an effective role in the development of performance for a man-to-man defense and the ratio of possession, by increasing the desire and motivation towards training and highlighting the potential capabilities of the players, as (1) indicates that the style of play one of the methods that have a clear impact and are used by coaches frequently in different periods of preparation, because they seem easy-leaning and this is evident in football by giving duties towards the goal to be achieved during the course of playing in various forms, and this method is characterized by the development of physical, skill and planning elements 6. The researchers also see that the use of these exercises within specific areas in the training units was a clear goal, which facilitated the work of the players, and that the repetition of performance was for the purpose of ensuring accuracy and speed in performance and avoiding errors during the performance of the players, and this was confirmed (2). That the repetition of exercises in the training unit makes the players less wrong in the implementation of duties and more control over performance, whether with the ball or without the ball, which makes the player take the correct position to perform the skillful or planned duty and then avoid the mistakes that occur in it as it gains the player good compatibility for performance.

Also the researchers see that group training within the specified spaces has an effective effect in spreading the spirit of cooperation and continuous work among the members of the same group and works to raise the player's skill and planning capabilities, both defensive and offensive, as (3), indicates that group training is the point The basic exercises in the exercise and that at the same time it develops the movement and planning skills of the players.
Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached:

1- Pressing style play exercises developed man-to-man defense, with both types of close and flexible defense for the experimental group under study.

2- The experimental group surpassed the control group in the post test and in all research variables.

3- Pressing play exercises performed a great role in strengthening the bonds of teamwork among the players in terms of bearing some of the mistakes received and covering them up.

Recommendations:

1- Teaching and training novices and juniors the style of stress playing, in a way that suits each stage, as a basis for them in the future.

2- The use of a stress playing style in the development of other types of individual or team defense.

3- Training the style of pressing play according to different playing areas.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Physical Education and Sports Sciences and all experiments were carried out in accordance with approved guidelines.

References


3. Al-Hiti MA. Tests and Tactics in Football, Amman, Dar Degla, 2007; 76.


Assessment of Mothers' Knowledge about Febrile Convulsions of Children at Ibn- Albalady Hospital in Baghdad City

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Lecturer, Pediatric Nursing Unit, College of Nursing, Al-Bayan University, Baghdad / Iraq

Abstract

Background: In terms of occurrence, about of children in the age group of one to five years have at least one episode of febrile convulsion. These, have recurrent seizures and many get admitted to the hospital.

Aims: This study was conducted to assess the level of mother's knowledge about Febrile Convulsions

Methodology: A descriptive study design has been conducted with pediatric emergency unit at the Ibn-Albalady Hospital in Baghdad City during the period from the 3rd March 2020 up to the 13th 2020. A purposive non probability sample of (100) mother were selected from emergency pediatric unit. Out of the women questioned, febrile convulsion was reported by 100 mothers. Table (2) summarizes that the mothers knowledge items concerning child with febrile convulsion. In general, their knowledge were poor to fair in all items, and they are accounted for 2(18.1%), 9(81.9%) respectively, and overall mothers knowledge about febrile convulsion were fair. The results illustrate that (48%) of the study sample represent fair knowledge, (39%) were having poor information and (13%) were having good information about child with febrile convulsion at emergency ward of Ibn- Albalady hospital. The data were collected by using constructed questionnaire, which consisted of 11 items self-administrated method used and filled by using the questionnaire. Been described of data analyzed through using of two statistical approaches. Descriptive statistical analysis and inferential statistical analysis.

Results: The study results revealed that (48%) of the mothers under study were having fair knowledge. Mothers' knowledge regarding febrile seizures was non-significantly associated with positive history of febrile seizures, urban residence, higher parental education, and working mothers.

Conclusions: Generally, mothers’ knowledge regarding their children with febrile convulsions was poor to fair.

Keywords: Mothers’ Knowledge, Febrile Convulsions, Children

Introduction

A febrile convulsions, or febrile seizures, are frequently encountered in pediatrics, and despite often being self limiting, these seizures strike fear in the hearts of patients' careers. Febrile seizure is the most common type of convulsive disorder and one of the most recorded causes of emergency hospital admission in children under 5 years of age In spite of having a good prognosis, FS is extremely frightening, emotionally traumatic, and anxiety provoking when witnessed by parents, which make it a very difficult condition for them to deal with. Concerns about the future health of the child are the most common causes of fear among the parents. Sources of concern include fear of recurrence, physical disabilities, mental retardation, and even death. During seizure, the parents may think that their child is dying, but fortunately, most of febrile convulsions are benign rarely have FSs caused brain damage and except developing countries, there are no documented cases of febrile convulsions-related deaths on record. A febrile convulsion was defined as an event in infancy or childhood, usually occurring between three months and five years of age, associated with fever but without evidence of intracranial infection or a defined cause. Many parents think that their child is dying when he or she has a febrile convulsion, and they are concerned that epilepsy or mental retardation may result.
Febrile convulsions are common, occurring in 2 to 4 percent of children at least once before five years of age. Mental retardation has been reported in up to 22 percent of children with febrile convulsions who were hospitalized or seen in specialized clinics., found that children who had febrile convulsions did not differ in intelligence from their normal seizure-free siblings at seven years of age.

Febrile seizure (FS) is a benign convulsive disorder in under 5-year-old children, but at the same time, it is an alarming event in the lives of both child and parents. Lack of parent's knowledge about the nature of FS and how they should deal with it can lead to poor management.

The fever is one of the most common presenting complaints, is being the reason for health visits in about 70% of pediatric age group. Parents frequently perceive fever as a disease rather than a symptom or sign of illness.

Studies found that parents are not correctly informed or well educated about the definition, measurement, and diagnosis of fever.

**Objectives:** To assess the knowledge of mothers regarding febrile seizure in their children under five years

**Methodology:** A descriptive study design was conducted with pediatric emergency unit at Ibn-Albalady Hospital in Baghdad City from the 3rd March 2020 up to the 13th 2020. A purposive non probability sample of (100) mother were selected from emergency pediatric unit. The study instrument consisted of two parts: First part related to characteristics of studied mothers and children seizure. Second part related to mothers knowledge data about their children with febrile seizure. The scoring system for the questionnaire was dichotomous, with the correct answer scoring (1) and the incorrect answer scored zero (0). The data were collected by using the constructed questionnaire, which consisted of 11 items self-administrated method used and filled by using the questionnaire. Been described of data analyzed through using of two statistical approaches. Descriptive statistical analysis and inferential statistical analysis.

**Results and Discussion**

<table>
<thead>
<tr>
<th>No.</th>
<th>Variables</th>
<th>Groups</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Child Age Groups (years)</td>
<td>0–1</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2–3</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3–4</td>
<td>20</td>
<td>20</td>
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<tr>
<td></td>
<td></td>
<td>4–5</td>
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<td>Child Gender</td>
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<td>40</td>
</tr>
<tr>
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<td></td>
<td>Total</td>
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<td>100</td>
</tr>
<tr>
<td>3.</td>
<td>Age group Groups (years)</td>
<td>18–22</td>
<td>21</td>
<td>21</td>
</tr>
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<td></td>
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<td></td>
<td>28–32</td>
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<td></td>
<td>≥ 33</td>
<td>24</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
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<td>100</td>
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<td>Mother Education level</td>
<td>Illiterate</td>
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<td>9</td>
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<tr>
<td></td>
<td></td>
<td>Primary school</td>
<td>10</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>Secondary school</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diploma or Bachelor’s degree</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
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<td></td>
<td>Higher education</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
<td>100</td>
<td>100</td>
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</table>

Table (1): Characteristics of mothers and their children with febrile convulsion
### Table (1): Characteristics of mothers and their children with febrile convulsion

<table>
<thead>
<tr>
<th></th>
<th>Mother occupation</th>
<th>House wife</th>
<th>45</th>
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<tr>
<td></td>
<td>Employed</td>
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<td></td>
<td>Total</td>
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<td>100</td>
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<table>
<thead>
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<th></th>
<th>The child’s age at the first occurrence of febrile convulsion</th>
</tr>
</thead>
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<tr>
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<td>Less than 1 year</td>
</tr>
<tr>
<td></td>
<td>1-2 years</td>
</tr>
<tr>
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<td>3 years and more</td>
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<tr>
<td></td>
<td>Total</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Duration of convulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Less than 5 minutes</td>
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<tr>
<td></td>
<td>5 to less than 15 minutes</td>
</tr>
<tr>
<td></td>
<td>More than 15 minutes</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

$F$: frequency, $%$: percentage

### Table (2): Distribution of mothers’ knowledge responses regarding items of febrile convulsion

<table>
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<tr>
<th>List</th>
<th>Knowledge items</th>
<th>Resp.</th>
<th>F</th>
<th>%</th>
<th>MS</th>
<th>SD</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Febrile seizure is a form of epilepsy.</td>
<td>False</td>
<td>68</td>
<td>68.0</td>
<td>.32</td>
<td>.469</td>
<td>Poor</td>
</tr>
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<td></td>
<td></td>
<td>True</td>
<td>32</td>
<td>32.0</td>
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<tr>
<td>2.</td>
<td>Anticonvulsant drugs are required for every FS child.</td>
<td>False</td>
<td>52</td>
<td>52.0</td>
<td>.48</td>
<td>.502</td>
<td>Fair</td>
</tr>
<tr>
<td></td>
<td></td>
<td>True</td>
<td>48</td>
<td>48.0</td>
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</tr>
<tr>
<td>3.</td>
<td>Every FS child will have another FS.</td>
<td>False</td>
<td>58</td>
<td>58.0</td>
<td>.42</td>
<td>.496</td>
<td>Fair</td>
</tr>
<tr>
<td></td>
<td></td>
<td>True</td>
<td>42</td>
<td>42.0</td>
<td></td>
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</tr>
<tr>
<td>4.</td>
<td>FS is rare after the age of 5 years.</td>
<td>False</td>
<td>61</td>
<td>61.0</td>
<td>.39</td>
<td>.490</td>
<td>Fair</td>
</tr>
<tr>
<td></td>
<td></td>
<td>True</td>
<td>39</td>
<td>39.0</td>
<td></td>
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<tr>
<td>5.</td>
<td>Recurrence FS will cause brain damage.</td>
<td>False</td>
<td>50</td>
<td>50.0</td>
<td>.50</td>
<td>.503</td>
<td>Fair</td>
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<td></td>
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<td>True</td>
<td>50</td>
<td>50.0</td>
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<tr>
<td>6.</td>
<td>Risk of subsequent epilepsy in FS children is rare.</td>
<td>False</td>
<td>57</td>
<td>57.0</td>
<td>.43</td>
<td>.498</td>
<td>Fair</td>
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<td>True</td>
<td>43</td>
<td>43.0</td>
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<tr>
<td>7.</td>
<td>It is necessary to put protective devices into the mouth to prevent tongue injury during convulsion.</td>
<td>False</td>
<td>60</td>
<td>60.0</td>
<td>.40</td>
<td>.492</td>
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</tr>
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<td>True</td>
<td>40</td>
<td>40.0</td>
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<tr>
<td>8.</td>
<td>It is necessary to restrain the child to stop the seizure during convulsion.</td>
<td>False</td>
<td>62</td>
<td>62.0</td>
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<td>.488</td>
<td>Fair</td>
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<td>True</td>
<td>38</td>
<td>38.0</td>
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<tr>
<td>9.</td>
<td>It is necessary to do mouth-to-mouth resuscitation during convulsion.</td>
<td>False</td>
<td>66</td>
<td>66.0</td>
<td>.34</td>
<td>.476</td>
<td>Fair</td>
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<td></td>
<td></td>
<td>True</td>
<td>34</td>
<td>34.0</td>
<td></td>
<td></td>
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<tr>
<td>10.</td>
<td>Children with febrile seizures will not have normal school achievement</td>
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<td>42</td>
<td>42.0</td>
<td>.58</td>
<td>.496</td>
<td>Fair</td>
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<td>True</td>
<td>58</td>
<td>58.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11.</td>
<td>EEG or CT is necessary for every FS child.</td>
<td>False</td>
<td>76</td>
<td>76.0</td>
<td>.24</td>
<td>.429</td>
<td>Poor</td>
</tr>
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<td></td>
<td>True</td>
<td>24</td>
<td>24.0</td>
<td></td>
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</tbody>
</table>

Grande mean of score = 0.41

$F$: frequency, $%$: percentage, $SD$: Standard Deviation, $MS$: mean of score, $Ass.$: Assessment by $MS$: (0.00 – 0.33) Poor; (0.34 – 0.66) Fair; (0.67– 1) Good.
Table (2) summarizes the mothers knowledge concerning items of febrile convulsions, in general, their responses were poor to fair in all items, and they are accounted for 2(18.1%), 9(81.9%) respectively, and overall mothers knowledge about febrile convulsion were fair.

The experiences of mothers whose children suffered from FC were complicated. The findings indicated that the experiences of these mothers were reflected in three themes, namely perceived threat, seeking solution, and difference in adaptation ⁹.

In terms of perceived threat, mothers believed that although FC often had a good prognosis and usually had no serious complications, the disease was still a threat to the child. Observing children during convulsion was reported as a scary event by mothers. They talked about their concerns about growth and development disruptions in the future life of their child. Studies have shown that seizures in children created serious concerns and fear in parents.² Fear of death, repeated seizures, low IQ, mental retardation, learning disabilities, paralysis, physical disability and an uncertain future for the child were some sources of fear for parents ¹⁰.

The results showed that mothers tried to find the best treatment and care for their child in order to restore her/his health. They also constantly searched for information in order to learn how to care for their child, so as to prevent relapsing convulsions and their complications. It was found that fever and FC were particularly worrisome for parents, causing them to frequently seek medical advice. They often required information which could enable them to manage their child’s illness ¹¹.

The results in figure (1) illustrate that (48%) of the study sample represent fair knowledge.

This table shows (48.%) of the sample was fair,(39%) were poor information and (13%) were good information of mothers about child with febrile convulsion at emergency ward of the non-teaching hospital.

Figure (1): Distribution of the mothers’ knowledge about febrile convulsion according to levels of assessment.
One of the necessary supports for parents was informational support. Such support includes the information delivered by healthcare personnel and the individuals in close contact with the child. Overprotecting the child was seen in some mothers. They felt that they have to monitor the child frequently to protect her/him. Several studies have shown that some sick children were under excessive control and monitoring.  

Also, a study found that the parents of children who suffered from chronic sicknesses not only accepted their children’s condition and its concomitant limitations, but also tried to remain optimistic about the future and hoped for the emergence of future effective medical treatments for the disease.  

Was found. Mothers who have a background with FS tended to be employed, have a higher education, and have urban residence compared to their non-working, rural, poorly educated counterparts. Regarding the attitude of the mothers towards FS, it was seen that although mothers can recognize a FS, a much lower percentage showed the ability to see the signs that precede the convulsions such as (only 38.3% knew that FS could reoccur.  

A large percentage knew about reducing the temperature, putting the child in safe place, placing the child in a lateral position. However, many mothers showed that they were doing incorrect practices such as opening the mouth and placing something inside, shaking the child, suction of secretions from the child’s nose and mouth, doing cardiac massage, and stimulation of the child. While earlier it was apparent that 91.6% of mothers knew that it was important to constantly monitor a child’s temperature, This goes to show that while these mothers may have good understanding of the condition; short, consistent reminders via the media or the public health sector (posters, advertisements) can raise the needed awareness to allow for a better understanding and attitude towards FS.  

All these items revealed bad knowledge which mearf bad quality of care provided to child with febrile convulsion. These results need an important gain steps to improve the care which help the major areas of advancement in child care include fever resuscitation protocols, early care to prevented of the febrile econvulsion, nutritional support regimens, topical antimicrobials and infection control, treatment of sepsis, thermally-neutral environments, and pharmacological modulation of the hyper-metabolic response. These factors have contributed to improved of child health care, reduced inflammation and energy demands, attenuated hyper-metabolism and muscle catabolism, and consequently decreased morbidity and mortality following and complicatio of high fever.  

This result of the study indicated that most of mothers have fair of knowledge toward information care of child with febrile convulsion. They worked routine for child with FC at home. The knowledge of standard care is not performed by large number of mothers which is due to many causes: the different level of education of mothers may affect their checking and readings of the vital signs, the preparation and using equipments that is used in Providing care as measurement of vital signs which is not enough lack of interventions related to inadequate structural continues education Programs as a result of hospitals’ policy and policy of Ministry of Health for continuing of education, insufficient nursing resources like library, online resources, reading of journals and lack of mothers documentation.  

A mother who has witnessed her child’s seizure and hospitalization is emotionally under a lot of pressure. She is always afraid of complications and recurrence of the event. Nurses in a family-centered profession are in such an excellent place to support mothers and families in coping with the situation and providing them with sufficient information in order to help them to gain a better understanding of the disease and also prevent its possible recurrence.  

Conclusions  

Generally, mothers’ knowledge regarding their children with febrile convulsions was poor to fair.  

Recommendations: Health education activities need to be focused on increasing mothers’ knowledge through various channels and assuring on double activities whether individually or in groups and further research can be carried out on large sample size of mothers with focus on variety of related variables that may influence their knowledge concerning child with febrile convulsion.
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Bayan University and all experiments were carried out in accordance with approved guidelines.

References
Is there an Impact of PAI-1 on the Thrombotic Episode in Iraqi Obese Patients with Corona Virus -19

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Abstract

Objective: firstly To assess the incidence of Corona Virus -19 covid-19 as risk factors in obese individuals leading to thrombotic episode, and secondly to compare the effects Plasminogen activator inhibitor-1 (PAI-1) on the occurrence of this thrombotic status in obese and normal body weight individuals. The pathogenesis of Coronavirus disease 2019 (COVID-19) is gradually demonstrated and explained around the world A high and a great number of thrombotic episodes are reported, in present study can be consider the COVID-19 as a prothrombotic disease.

Design and Methods: The experiments comparing the PAI-1 in 90 obese and 30 normal body weight participants aged 32-62 years. The obese patients subdivided to three groups: first group consisted from 30 over weight individuals and the second group comprised from 30 obese patients, while third group consisted from 30 morbid obesity patients. All participants underwent to the medical examinations to make sure they were infected with covid-19. The activity of PAI-1 and tissue plasminogen activator (t-PA) was determined by applied ELISA method by certain kit in all study groups individuals.

Results: Three study groups (overweight, obese, and morbid obesity) elicited significant (p< 0.05) elevation in metabolic characteristics such as fasting blood glucose (FBG), fasting insulin and lipids variables, as well as increasing in insulin resistance (HOMA-IR) and D-dimer fractions, in addition to there were significant augmentation in PAI-1 and t-PA in three study groups when comparing with those of normal or healthy body weight

The present results show a positive relationship between PAI-1 and body mass index, t-PA, D-dimer and metabolic characteristics which include fasting blood glucose, fasting insulin and lipids fractions, also HOMA-IR.

Key Words: Covid-19, Obesity, PAI-1, t-PA, thrombosis

Introduction

Coronaviruses are a great family of viruses which cause diseases in animals and humans. Seven coronaviruses can leading to infection in people around the world but commonly people have infected with these four human coronaviruses 1. The main organ which affected is a respiratory system, the infection ranging from the common cold to more severe comorbidities such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) and the most recently discovered coronavirus (COVID-19) causes infectious disease 1. The virus is spread rapidly between individuals through respiratory droplets during coughing. As of 13 September 2020, 210 Countries and Territories around the world have reported over 28,946,628 confirmed cases and 924,610 deaths of COVID-19, only in Iraq show 286,778 coronavirus cases and 7,941 deaths.

There are very few available data on impact of (body mass index) BMI for patients with COVID-19 infections, the obesity in the COVID-19 epidemic plays a major role in the pathogenesis of COVID-19 infection. It is believed the immune system, which is an essential player in the pathogenesis of COVID-19, also plays an substantial role in obesity which enhanced inflammation.
in adipose tissue. The metabolic dysfunction is a main cause of inflammation in adipose tissue which potentially leading to dyslipidaemia, insulin resistance, diabetes mellitus, hypertension, and cardiovascular disease CVD . In previous research the case of influenza A, was the obesity increases the duration of virus and severity ; the complication of having virus in patients with obesity was 42% longer than persons who do not have obesity . Another study indicated that in H1N1 influenza, obesity is consider as risk factor for hospitalization and death .

Several investigators revealed that BMI was significantly higher in patients with a severe form of COVID-19 infection . The published of retrospective analysis on 112 patients with COVID-19 infection demonstrated that BMI of the critical group with range (23.0-27.5 kg/m²) was significantly higher than that of the general group with range( 20.0-24.0 kg/m²), then critical group were subdivided into two groups, survivors (84.8%) and non-survivors (15.18%). Among the non-survivors, 88.2% of patients had a BMI > 25 kg/m² . Thrombotic events were an aggravating cause of death. Thromboembolic risk is known to be higher in patients with obesity than in the general population. It logically follows that obesity can be an aggravating risk factor for death from COVID-19 infection .

Plasminogen activator inhibitor-1 (PAI-1) play an important role in inhibition of plasminogen activators which included tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), additionally, it regulates the fibrinolytic system as well as PAI-1 have a pivotal role in acute thrombotic phenomenon such as deep vein thrombosis (DVT) and myocardial infarction (MI). PAI-1 have more biological effects beside to the role in thrombosis including its central role in fibrotic disorders, atherosclerosis, renal and pulmonary fibrosis, diabetes mellitus, and cancer .

PAI-1 is produce in adipose tissue, and its levels in plasma are increased in obesity and reduced with weight loss . In a great epidemiological studies, the high level of PAI-1 has been established as a predictor of myocardial infarction . It is important to mention that effect of PAI-1 eliminates after adjustment metabolic syndrome components .

Abnormalities in coagulation factors and imbalance illustrated the link between obesity and thrombosis which occur in both arterial and venous . Several studies have shown that patients with BMI higher than 30 kg/m² have higher concentrations of all pro-thrombotic factors (fibrinogen, vonWillebrand factor (vWF), and factor VII), when compared to normal BMI controls, with a positive correlation with central obesity, in the same way, the concentrations of plasminogen activator inhibitor-1 (PAI-1) have been elevated levels in obese patients when compared with non-obese controls, as well as, it is correlated directly with visceral fat. Likewise , patients with obesity are characterized by higher plasma levels of anti-thrombotic factors, which include, tissue-type plasminogen activator (t-PA) and protein C, when compared with non-obese individuals, the enhancing these factors may be represent a protective role to counteract the increase in pro-thrombotic factors . Just as mentioned earlier the adipose tissue participate directly in product of plasma PAI-1, then stimulating other cells to produce PAI-1. In addition to, the adipose tissues secreted interleukin-6 (IL-6) which linked with the actions to produce tumor necrosis factor-α (TNF-α) in obesity, these findings could explain the association of insulin resistance with endothelial dysfunction, coagulopathy, and coronary heart disease. Recent studies mentioned the role of leptin in impairing balance and enhancing thrombosis, so the abnormalities value of some hormones such as (androgen, catecholamines) likely to combined with the accumulation of body fat may be leading to the impairment of coagulative pathway in obesity . Some investigators demonstrated to be efficient method to improving the obesity combined with pro-thrombotic risk profile .

![Figure (1): The role of PAI-1 in inhibition of fibrinolysis](image-url)
Systemic inflammation, endothelial dysfunction, disturbances of lipid and glucose metabolism, and IR contribute to the hypercoagulable state and the impaired fibrinolysis found in obesity. However, obesity is characterized by the elevation of several clotting factors and PAI-1 directly affecting coagulation and fibrinolysis independent of genetic factors. Moreover, a recent study has reported that subcutaneous adipose tissue shows a stronger relationship with functional measures of hypercoagulability as compared to visceral adipose tissue, suggesting that the anatomic location of adipose deposition may influence the type of thrombotic event, with visceral adipose tissue being associated with arterial thrombosis whereas subcutaneous adipose tissue predisposing to venous thrombosis.

Materials and Methods

Study Design

In this study, 120 hospitalized individuals with covid-19 infection aged 32-62 years were recruited during the period April–May 2020. The 90 obese patients subdivided to three groups: first group consisted from 30 overweight individuals (BMI 25.0-29.9 kg/m²) and the second group comprised from 30 obese patients(BMI 30.0-39.9 kg/m²), while third group consisted of 30 extreme obesity patients(BMI >40 kg/m²) while the fourth group comprised from 30 covid-19 patients with healthy body weight (BMI 18.5-24.9 kg/m²). The questionnaire in the present study was prepared in accurately depending on the opinion of the specialist, including medical swap, D-dimers analysis and CT-scan in addition to the demographic characteristics such as height, weight, and vital signs such as systolic and diastolic blood pressure, all this information was provided through oral interviews with patients and supervising physicians.

Blood Biochemistry Analysis:

All blood sampling was carried out under sterile conditions. Blood glucose measurements which included fasting blood glucose, post prandial blood glucose using certain kits from Spinract, Spain, fasting insulin levels using ELISA kit from Calbiotech, USA then determined HOMA-IR by certain equation. In addition to the measurement of blood lipid profiles were carried out, that included total cholesterol, HDL-cholesterol and triglycerides were obtained between 8 and 10 am following a 10-h fast, using kits from Biolabo, France. Estimates of LDL-cholesterol were calculated using the Friedewald equation. Plasminogen activator inhibitor 1 (PAI-1), tissue-type plasminogen activator (t-PA), and D-dimers were measured using ELISA method by Elabscience, China kit.

Statistical Analysis

The statistical analysis was achieved by the statistical package for the social science (SPSS) software for windows, version 20.0. the result were represented as mean ± standard deviation (Mean ± SD). Two - way Analysis of variance was used to compare variables in different studied groups. Pearson’s correlation was applied to determine the relation among the measurable factors of the present study, significant was determined regression. The confidence interval was set at 95%, thus p values less than 5% (p<0.05) were considered statistically significant.

Result and Discussion

A total of 120 patients (77 males and 43 females) were participate in current study with a mean age of 51.2 ± 16.4. sixty -two patients were entered to intensive care unit ( ICU) ; most of them were obese or they have extreme obesity. Table (1) illustrated patient characteristics and laboratory data.

18 (29%) of ICU patients developed thrombosis (10 pulmonary embolisms, 6 deep vein thromboses, and 2 acute aortic thrombosis) diagnosed with computational tomography scan (CT scan) and other laboratory tests such as (D-dimers analysis). D-dimers were increased in 80% of overweight, obese, and extreme obesity patients when compared with healthy weight individuals, most of them were admitted to (ICU) in hospital. ICU patients from three groups (overweight, obese, and extreme obesity) presenting with an aggressive form of Covid-19 disease who had significantly higher levels of t-PA, PAI-1 (p=0.000) of each other when compared with ICU patients who had healthy weight.

Plasminogen activator inhibitor PAI-1 is the primary inhibitor of both tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators, which inhibits fibrinolysis and has causative relationship with various vascular complications.
In addition to PAI-1 is the dominant inhibitor of the fibrinolytic system. Increased concentration of PAI-1 in the circulation enhanced hypofibrinolysis which is a state of impairment of removal the thrombi from the vascular system, by degradation cellular matrix, migration of smooth muscle cell and angiogenesis, as well as PAI-1 may affect in the development of atherosclerotic lesions.

High PAI-1 levels are correlated with an cardiovascular risk of atherothrombosis, dyslipidemia, hyperinsulinemia, and hypertension. PAI-1 has important role in acute thrombotic events such as fibrotic disorders including atherosclerosis and renal and pulmonary fibrosis. Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) directly effect thrombus formation and degradation leading to risk for arterial thrombosis. PAI-1 is a procoagulant, proinflammatory, and profibrotic molecule. The PAI-1/tissue plasminogen activator (tPA) ratio uses as a tool of a patient’s fibrinolytic balance which can indicate thrombus and stroke risk.

The present study finding agreed with the study which mentioned that levels of (t-PA) are increased in obese diabetic patients and the major episode behind atherosclerosis is inhibition of fibrinolysis due to enhanced plasminogen activator inhibitor-1 (PAI-1) levels, marker of ineffective fibrinolysis leading to increased thrombus formation and produce the unstable plaque. Additionally high serum glucose concentration also has been shown to be associated with elevated PAI-1 levels. Increased PAI-1 levels have been encountered in many disease conditions, including metabolic syndrome, diabetes, and obesity. The results of the prospective cardiovascular study indicated that PAI-1 play important role in adipose tissue development and insulin signaling in adipocytes. PAI-1 is the primary inhibitor of both tissue-type and urokinase-type plasminogen activators, which inhibits fibrinolysis and has causal relationship with various vascular complications.

The outcomes showed a significant increase in blood sugar levels and insulin concentration in overweight, obese and extreme obesity patients comparing with healthy weight patients, the aim of evaluate fasting blood sugar and fasting insulin was to determine the insulin resistance by certain equation by HOMA-IR formula, table (1) shows highly significant increase in HOMA-IR values in obese and extreme obesity groups, while did not show significant differences between obesity and extreme obesity.

The study reported a significant increase in the levels of triglycerides, and very low density lipoprotein binding cholesterol (vLDL-c) of patients with overweight, obesity and morbid obesity comparison to healthy weight individuals group. In addition to the present study shows that high density lipoprotein binding cholesterol (HDL-C) in serum of obese and extreme obesity groups is significantly decreased (p<0.05) compared with that of healthy weight group show Table (1).

### Table (1): Levels (Mean ± SD) of characteristics and some laboratories biomarker in Sera of Studied Groups

<table>
<thead>
<tr>
<th>variables</th>
<th>Healthy weight</th>
<th>Over weight</th>
<th>Obese</th>
<th>Extreme obesity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI kg/m2</td>
<td>23.56±10.23</td>
<td>28.54±12.66</td>
<td>36.43±12.76</td>
<td>44.56±14.34</td>
<td>0.070 For 1 vs 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000 For 1 vs 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000 For 1 vs 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003 For 2 vs 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000 For 2 vs 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.012 For 3 vs 4</td>
</tr>
<tr>
<td>Fasting blood glucose mg/dL</td>
<td>86.54±15.51</td>
<td>120.60±15.59</td>
<td>130.23±69.98</td>
<td>135.61±81.24</td>
<td>0.010 For 1 vs 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000 For 1 vs 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000 For 1 vs 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.063 For 2 vs 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.000 For 2 vs 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.153 For 3 vs 4</td>
</tr>
</tbody>
</table>
**Cont.. Table (1): Levels (Mean ± SD) of characteristics and some laboratories biomarker in Sera of Studied Groups**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>p-value (1 vs 2)</th>
<th>p-value (1 vs 3)</th>
<th>p-value (1 vs 4)</th>
<th>p-value (2 vs 3)</th>
<th>p-value (2 vs 4)</th>
<th>p-value (3 vs 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin μIU/L</td>
<td>14.45±3.65</td>
<td>18.77±5.78</td>
<td>24.89±8.35</td>
<td>27.83±8.80</td>
<td>0.061</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.173</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.41±0.578</td>
<td>2.34±1.123</td>
<td>3.01±1.45</td>
<td>3.33±1.366</td>
<td>0.070</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.032</td>
<td>0.353</td>
</tr>
<tr>
<td>Triglyceride mg/Dl</td>
<td>133.33±300.24</td>
<td>169.92±85.00</td>
<td>228.75±90.11</td>
<td>235.55±48.98</td>
<td>0.045</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.353</td>
<td>0.193</td>
</tr>
<tr>
<td>HDL mg/Dl</td>
<td>68.25±22.89</td>
<td>43.67±17.59</td>
<td>34.89±6.45</td>
<td>33.95±8.05</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.013</td>
<td>0.653</td>
</tr>
<tr>
<td>vLDL mg/Dl</td>
<td>38.40±7.19</td>
<td>45.39±16.50</td>
<td>66.60±18.03</td>
<td>67.32±20.09</td>
<td>0.060</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.023</td>
<td>0.754</td>
</tr>
<tr>
<td>PAI-1 ng/mL</td>
<td>52.67±10.28</td>
<td>74.67±30.18</td>
<td>82.56±39.34</td>
<td>96.35±35.13</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.053</td>
<td>0.050</td>
</tr>
<tr>
<td>t-PA ng/mL</td>
<td>14.82±7.57</td>
<td>22.97±11.86</td>
<td>43.9±19.5</td>
<td>61.44±30.54</td>
<td>0.067</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.905</td>
</tr>
<tr>
<td>D-Dimers ng/mL</td>
<td>798.8±398.8</td>
<td>864.6±439.2</td>
<td>3412.7±1332.5</td>
<td>3677.5±1641.2</td>
<td>0.047</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.905</td>
</tr>
</tbody>
</table>
1: Healthy weight 2: Over weight 3: Obese 4: Extreme obesity

As shown in Table (2), serum (PAI-1) concentration were positively correlated with BMI, fasting blood glucose, fasting insulin, HOMA-IR, triglyceride, VLDL, t-PA, and D-dimers, while inversely correlated with HDL, these correlation indicated (PAI-1) levels effecting on the independent risk factors which proved that (PAI-1) might be participated in thrombosis occurrence.

Previous studies provides more evidences that PAI-1-dependent mechanisms may attribute to the pathogenesis of insulin resistance and type 2 diabetes mellitus. For example, in the IRAS study (Insulin Resistance Atherosclerosis Study, a longitudinal cohort of 1047 subjects followed for 5 years), PAI-1 was a mainly predictor for the evolvement of diabetes mellitus, even after adjusting for adipose tissue distribution, and insulin sensitivity. Another study revealed that visceral and subcutaneous adipose tissue PAI-1 mRNA expression were positively correlated with BMI in severe obesity (r=0.043/0.56, respectively). Moreover, there are more information for that weight reduction virtually reduces plasma PAI-1 in obese humans. These previous findings are in line with present results which correlating serum PAI-1 with adipose tissue BMI. Furthermore, another study demonstrated that PAI-1 which secreted from omental adipose tissue is greater than that from subcutaneous adipocytes. Thus, increased omental fat secretion of PAI-1 may be contribute to the enhanced atherothrombotic risk of human central obesity.

In epidemiological studies, there are a direct link between PAI-1 and lipid parameters (e.g., triglyceride and HDL-C levels) in healthy young adults. Also, recent studies supposed that very low density lipoprotein (VLDL) was responsible for increasing the PAI-1 level by increasing a VLDL response element localized to the promoter region of the PAI-1 gene and mediating VLDL which induced PAI-1 transcription in endothelial cells.

A previous in vitro research also showed that small-sized HDL, but not large-sized HDL, enhanced PAI-1 release in the murine 3T3 adipocyte cell line; however, to date, there are limited data on the association of PAI-1 with the functional and structural properties of the different lipoprotein fractions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PAI-1 Healthy Weight</th>
<th>PAI-1 Over weight</th>
<th>PAI-1 Obese</th>
<th>PAI-1 Extreme obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.248</td>
<td>0.525**</td>
<td>0.785**</td>
<td>0.825**</td>
</tr>
<tr>
<td></td>
<td>0.079</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood Glucose</td>
<td>0.058</td>
<td>0.189</td>
<td>0.463**</td>
<td>0.567**</td>
</tr>
<tr>
<td></td>
<td>0.653</td>
<td>0.194</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.012</td>
<td>0.155</td>
<td>0.459**</td>
<td>0.659**</td>
</tr>
<tr>
<td></td>
<td>0.893</td>
<td>0.284</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.250</td>
<td>0.760**</td>
<td>0.431**</td>
<td>0.731**</td>
</tr>
<tr>
<td></td>
<td>0.080</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.120</td>
<td>0.691**</td>
<td>0.622**</td>
<td>0.822**</td>
</tr>
<tr>
<td></td>
<td>0.420</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.071</td>
<td>0.619**</td>
<td>0.719**</td>
<td>0.881**</td>
</tr>
<tr>
<td></td>
<td>0.660</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.011</td>
<td>0.340*</td>
<td>0.431</td>
<td>0.531</td>
</tr>
<tr>
<td></td>
<td>0.949</td>
<td>0.016</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t-PA</td>
<td>0.111</td>
<td>0.382*</td>
<td>0.664**</td>
<td>0.860**</td>
</tr>
<tr>
<td></td>
<td>0.481</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.

The pathophysiology of Covid-19-related thrombosis is incompletely understood. In Covid 19 the Inflammation status promotes releasing tPA and PAI-1 from endothelial cells. In addition, the ctivation of platelets in covid-19, also release large amounts of PAI-1 because the platelets are the major circulating pool of PAI-1 that can contribute to a high local concentration of PAI-1 at the location of a growing fibrin clot. Increased PAI-1 is responsible for hypofibrinolysis and fibrin persistence. Interestingly, increased PAI-1 plasma levels were observed in patients during the SARS-CoV epidemic in 2002. Persistent fibrin deposition in lung parenchyma and alveolar spaces of Covid-19 patients strongly suggests that despite increased levels of tPA, high PAI-1 levels can overcome local tPA release. In addition, plasma hypofibrinolysis from elevated levels of PAI-1 and TAFI is a risk factor for venous thrombosis.

**Conclusion**

There are much evidences about the impact of PAI-1 on thrombosis formation in covid-19 patients who suffered from three types of obesity and the sever of disease associated with increment of BMI. In addition to the analysis of our data established that the homeostasis between coagulation and fibrinolysis is not found in patients with Covid-19 infection, who suffered from hypercoagulability and hypofibrinolysis at the same time which correlated with high PAI-1 and increased t-PA levels. Additionally, in present study concluded this severity of covid-19 increase with increase the number of metabolic syndrome components such as hyperglycemia, hyperinsulinemia and dyslipidemia.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Kufa University and all experiments were carried out in accordance with approved guidelines.

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Molecular, Biochemical, and Phenotypic Identification of Phenol and Cresol Degrading Acinetobacter baumannii Strain Selected from Al-Rumaila Oil Well

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Abstract

Background: phenol and cresol are dangerous compounds that have harmful effects on aquatic life, plants and, many other organisms. Therefore, it is necessary to effectively eliminate these compounds to protect the environment.

Aim: The aim of this study is to select and characterize bacterial strain(s) from oil contaminated soils and testing its ability for biodegrading phenol and cresol.

Methods: Biochemical tests were performed in addition to morphological and molecular detection through 16S rRNA analysis to identify the selected strain after growing on Mineral salts medium (MM) containing phenol or cresol. The residual concentration of phenol and cresol was analyzed over the time period by means of the 4-aminoantipyrine assay.

Results: the selected strain was identified as Acinetobacter baumannii strain HILLA-1, and it was able to remove 100% of the phenol at all the used concentrations after 48 h. This strain was also able to degrade 100% of cresol at concentrations (25,50,75,100,200 ppm /L) and 99.37%, 88.69%, and 94.73% at concentrations (300,400,500 ppm /L) respectively after 48 h.

Conclusion: The selected and identified Acinetobacter baumannii strain HILLA-1 can provide a putative source for bioremediation of phenol and cresol contaminated environments.

Keywords: Acinetobacter baumanni, phenol, cresol, Biodegradation, 16S rRNA

Introduction

A large number of organic compounds have been released to the environment since the beginning of the industrial revolution. These compounds are chemically manufactured and many of them are discarded as waste. Phenol is one of the most common toxic environmental pollutants, and it is classified as a dangerous substance to human health and other organisms (EPA, 2003) because many of phenolic compounds possess the ability to survive and not disintegrate in the environment. They also have the ability to move, biotransform, and bioaccumulate in human and animal tissues as well as increasing their concentration in the food chain.
and waste. Phenolic compounds have harmful effects on aquatic life, plants and many other organisms. The natural sources of phenol that were formed as a result of forest fires and the natural emission of phenol when asphalt was used as an adhesive in cities. On the other hand, cresol is a colorless viscous liquid that is used as an intermediate in the production of other chemicals. Its chemical formula is C7H8O. Methyl phenol is an aromatic organic compound that has a variable melting point that is determined by ambient temperatures. It is involved in various fields in the manufacture of pesticides, petroleum products, dyes, and also in the pharmaceutical industries. It is very toxic to humans if inhaled or swallowed, even in very low concentrations. It often results in problems such as eye, mouth, throat and skin irritation, vomiting, liver and heart damage, paralysis, coma, and sometimes death. Residues of phenol and its derivatives can be removed from the environment through a combination of physical and chemical treatments such as adsorption, osmosis, adsorption, photo catalysis, and electrolyte oxidation, but these treatments are impractical due to the high cost and formation of other toxic compounds. Therefore, biological methods have received more attention than physical and chemical methods because many different bacteria are known to use phenolic compounds as the only sources of carbon and energy. The degradation of phenol and its derivatives has been studied by bacteria. A large number of different bacterial species have been found to have the ability to biodegrade phenolic compounds such as Acinetobacter spp. In the current study, an Acinetobacter baumannii strain that has the ability to biodegrade phenol and cresol at high concentrations was selected from oil contaminated soil of Al-Rumaila oil well and identified based on phenotypic, biochemical, and molecular approaches. The Biodegradation rates of phenol and cresol by the selected strain were also reported in this study.

Materials and Methods

Sample collection

(20 samples) collected from soil contaminated with petroleum products from various places. Samples were collected in sterile glass bottles and transferred to the laboratory for the required tests.

Growth media

Mineral salts medium (MM) was used for selection. It consists of the following g/L materials, according to

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mgso 4.7H2O</td>
<td>(0.1g)</td>
</tr>
<tr>
<td>(2SO4)</td>
<td>(0.01g)</td>
</tr>
<tr>
<td>NaCl</td>
<td>(0.01g)</td>
</tr>
<tr>
<td>CaCl2</td>
<td>(0.45g)</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>(0.002g)</td>
</tr>
<tr>
<td>FeCl3</td>
<td>(16)</td>
</tr>
</tbody>
</table>

The materials were dissolved in a liter of distilled water and mixed well to ensure that all the materials were dissolved and sterilized for 20 minutes at 121 °C and the pH was adjusted (7).

Selection of phenol and cresol tolerant bacterial strains

A weigh of 5 g of each soil sample was added to 50 ml of liquid mineral salts medium (MM). Phenol or cresol was added at a concentration of (25 mg/L) and (25 ppm/L), respectively as the sole source of carbon and energy. The flasks then were incubated for 5 days at 30 °C. 0.1 ml of the bacterial suspension was transferred to MM agar medium containing the same concentrations of phenol and cresol and incubated for 5 days at 30 °C. This step was repeated two times to obtain pure colonies and to ensure that the isolated bacterial species are capable of degrading phenol and cresol.

The ability of the bacterial isolates to degrade phenol and cresol compounds

Standard titration curves were prepared for both compounds by preparing standard solutions for each of them. The assay of testing the ability of the isolated strain to degrade phenol (25, 50, 75, 100, 200, 300, 400, 500 mg/L) and cresol (25, 50, 75, 100, 200, 300, 400, 500 ppm/L) was performed as mentioned in (Barwick & Vicki 2003). Then using the absorption values on length 460nm to build a calibration curve for the two compounds using an Excel program to obtain the mathematical equation in order to calculate the concentrations of the remaining phenol and cresol compounds. After the growing of bacteria to assess their efficiency on length 600nm in the biodegradation process This assay was performed twice to confirm the degradation ability of the isolated strain to phenol and cresol twice to confirm the degradation of the isolate to phenol and cresol by 4-aminoantipyrine assay. Identification of the Phenol-cresol degrading Bacterium

The isolated bacterial strains was identified based on morphological characteristics on MacConkeyagar, Vitek 2 compact system biochemical tests (which was
used according to the manufacturer’s instructions), and sequence analysis of the 16S rRNA gene. DNA extraction and 16S rRNA sequencing. Genomic DNA was extracted from the selected Acinetobacter baumannii bacterial isolate using the PrepTM Genomic DNA MiniKit provided by Macrogen / Korea according to the manufacturer’s instructions. DNA amplification was performed by a PCR master mix containing 3 μl forward and reverse primers, 14 μl nuclease-free water, 5 μl Template DNA, 25 μl Master Mix. 16S rRNA gene was amplified from the DNA genome by PCR using the following forward and reverse primers for 16S rRNA respectively: 5’AGAGTTTGATCCTGGCTCA - 3’ and 5’GGTTACCTTGATTACGACTT -3. The polymerase chain reaction was carried out under the following conditions: Initial denaturation at 95 °C for 120 seconds followed by denaturation at 95 °C for 30 seconds, followed by annealing at 53 °C for 30 seconds, followed by elongation at a temperature of 72 °C for 150 seconds and the final elongation score of 72 for 300 seconds, respectively.

DNA sequencing analysis

The 16S rRNA PCR product of Acinetobacter baumannii strain HILLA-1 was extracted and sent to Bioneer (Korea) to perform nitrogenous base sequencing. The base sequence of the selected strain was registered in the NCBI GenBank records, and its accession number is MT032339.1. Using the BLASTn tool, Homologous sequences were attained from the NCBI database. Homology to Acinetobacter baumannii strain HILLA-1 were chosen with accession numbers: MK027249.1, MN636473.1, MN623687.1, MN175924.1, MN175925.1, MN175923.1, MN175922.1, MN175921.1, MN175920.1, MN173945.1. Sequences alignment and phylogenetic tree analysis were done using Mega X software. The phylogenetic tree was drawn based on neighbor-joining method.

Results

Characterization of the selected phenol/Cresol strain

An Acinetobacter baumannii strain, obtained in the current study, was isolated from oil contaminated soil of Al-Rumaila oil well. The strain was selected using a MM medium containing phenol or cresol then characterized after culturing on MacConkey agar plates. The colonies appeared small, round, convex and mucous, with a light cream color as shown in Figure 1. The colonies were purified and identified using Gram’s stain which showed Gram-negative bacterium in a form of semi-spherical bacilli. The selected strain was further subjected to biochemical identification using Vitek 2 compact system as shown in table 1.

Figure (1). Phenotypic characteristics of the isolated bacterial strain growing on solid mineral salts medium (A) and MacConkey agar medium (B).
Table 1: Biochemical properties of the isolated strain using Vitek 2 compact system.

<table>
<thead>
<tr>
<th></th>
<th>Compound</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>AlaPhe-Pro-ARYLAMIDASE</td>
<td>-</td>
<td>3</td>
<td>ADONITOL</td>
</tr>
<tr>
<td>4</td>
<td>L-Pyrrolydonyl-ARYLAMIDASE</td>
<td>+</td>
<td>5</td>
<td>L-ARABITOL</td>
</tr>
<tr>
<td>7</td>
<td>D-CELLOBIOSE</td>
<td>+</td>
<td>9</td>
<td>BETA-GALACTOSIDASE</td>
</tr>
<tr>
<td>10</td>
<td>H2S PRODUCTION</td>
<td>-</td>
<td>11</td>
<td>BETA-N-ACETYLC-GLUCOSAMINIDASE</td>
</tr>
<tr>
<td>12</td>
<td>GlutarylArylamidasepNA</td>
<td>-</td>
<td>13</td>
<td>D-GLUCOSE</td>
</tr>
<tr>
<td>14</td>
<td>GAMMA-GLUTAMYL-TRANSFERASE</td>
<td>+</td>
<td>15</td>
<td>FERMENTATION/GLUCOSE</td>
</tr>
<tr>
<td>17</td>
<td>BETA-GLUCOSIDASE</td>
<td>+</td>
<td>18</td>
<td>D-MALTOSE</td>
</tr>
<tr>
<td>19</td>
<td>D-MANNITOL</td>
<td>+</td>
<td>20</td>
<td>D-MANNITOL</td>
</tr>
<tr>
<td>21</td>
<td>BETA-XYLOSIDASE</td>
<td>+</td>
<td>22</td>
<td>BETA-Alanine arylamidasepNA</td>
</tr>
<tr>
<td>23</td>
<td>L-Proline ARYLAMIDASE</td>
<td>-</td>
<td>26</td>
<td>LIPASE</td>
</tr>
<tr>
<td>27</td>
<td>PALATINOSE</td>
<td>+</td>
<td>29</td>
<td>Tyrosine ARYLAMIDASE</td>
</tr>
<tr>
<td>31</td>
<td>UREASE</td>
<td>+</td>
<td>32</td>
<td>D-SORBITOL</td>
</tr>
<tr>
<td>33</td>
<td>SACCHAROSE/SUCROSE</td>
<td>+</td>
<td>34</td>
<td>D-TAGATOSO</td>
</tr>
<tr>
<td>35</td>
<td>D-TREHALOSE</td>
<td>+</td>
<td>36</td>
<td>CITRATE(SODIUM)</td>
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<tr>
<td>37</td>
<td>MALONATE</td>
<td>+</td>
<td>39</td>
<td>5-KETO-D-GLUCONATE</td>
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<tr>
<td>40</td>
<td>L-LACTATE alkalinisation</td>
<td>+</td>
<td>41</td>
<td>ALPHA-GALACTOSIDASE</td>
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<tr>
<td>42</td>
<td>SUCCINATE alkalinisation</td>
<td>+</td>
<td>43</td>
<td>Beta-N-ACETYLC-GLUCOSAMINIDASE</td>
</tr>
<tr>
<td>44</td>
<td>ALPHA-GALACTOSIDASE</td>
<td>+</td>
<td>45</td>
<td>PHOSPHATASE</td>
</tr>
<tr>
<td>46</td>
<td>Glycine ARYLAMIDASE</td>
<td>-</td>
<td>47</td>
<td>ORNITHINE DECARBOXYLASE</td>
</tr>
<tr>
<td>48</td>
<td>LYSINE DECARBOXYLASE</td>
<td>+</td>
<td>53</td>
<td>L-HISTIDINE assimilation</td>
</tr>
<tr>
<td>56</td>
<td>COUMARATE</td>
<td>+</td>
<td>57</td>
<td>BETA-GLUCORONIDASE</td>
</tr>
<tr>
<td>58</td>
<td>O/129 RESISTANCE(comp.vibrio.)</td>
<td>+</td>
<td>59</td>
<td>Glu-Gly-Arg-ARYLAMIDASE</td>
</tr>
<tr>
<td>61</td>
<td>L-MALATE assimilation</td>
<td>+</td>
<td>62</td>
<td>ELLMAN</td>
</tr>
<tr>
<td>64</td>
<td>L-LACTATE assimilation</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis of 16S rRNA gene sequence

16S rRNA fragment was amplified from the gDNA of the *Acinetobacter baumannii* isolate and detected with about 1498bp as shown in Figure 2. The sequence of 16S rRNA gene of the isolated strain of the current study can be found under the accession number MT032339.1 at NCBI–GenBank with the name of *Acinetobacter baumannii* strain HILLA-1. The BLAST results showed that the base sequence of the 16S rRNA of *Acinetobacter baumannii* strain HILLA-1 was 98.94% identical to *Acinetobacter baumannii* strain B18 (Accession number MK027249.1) and 98.82% identical to *Acinetobacter baumannii* strains with Accession numbers: MN636473.1, MN623687.1, MN175924.1, MN175925.1, MN175923.1, MN175922.1, MN175921.1, MN175920.1, MN173945.1 (Table 2).

Table 2: Homology sequence identity for 16S rRNA gene of *Acinetobacter baumannii* strain HILLA-1

<table>
<thead>
<tr>
<th>No.</th>
<th>Accession number</th>
<th>Name of species sequence</th>
<th>Identity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MK027249.1</td>
<td>Acinetobacter baumannii strain B18 16S ribosomal RNA gene, partial sequence</td>
<td>98.94</td>
</tr>
<tr>
<td>2</td>
<td>MN636473.1</td>
<td>Acinetobacter baumannii strain Charmo6 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>3</td>
<td>MN623687.1</td>
<td>Acinetobacter baumannii strain VGM2 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>4</td>
<td>MN175924.1</td>
<td>Acinetobacter baumannii strain DSM 1762 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>5</td>
<td>MN175925.1</td>
<td>Acinetobacter baumannii strain DSM 1924 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>6</td>
<td>MN175923.1</td>
<td>Acinetobacter baumannii strain DSM 1918 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>7</td>
<td>MN175922.1</td>
<td>Acinetobacter baumannii strain DSM 1923 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>8</td>
<td>MN175921.1</td>
<td>Acinetobacter baumannii strain DSM 1676 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>9</td>
<td>MN175920.1</td>
<td>Acinetobacter baumannii strain DSM 1675 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
<tr>
<td>10</td>
<td>MN173945.1</td>
<td>Acinetobacter baumannii strain rY32 16S ribosomal RNA gene, partial sequence</td>
<td>98.82</td>
</tr>
</tbody>
</table>
Phylogenetic tree analysis

Figure 3 shows the phylogenetic relationship analysis using the sequenced 16S rRNA gene from *Acinetobacter baumannii* strain HILLA-1 with all sequences listed in Table 2. The results revealed that *Acinetobacter baumannii* strain HILLA-1 is closely related to *Acinetobacter baumannii* strain rY32.

![Phylogenetic tree](image)

**Figure 3: Phylogenetic relationship of the sequenced 16S rRNA gene from *Acinetobacter baumannii* strain HILLA-1, accession number (MT032339.1). Analysis was done by neighbor-joining method.**

The ability of *Acinetobacter baumannii* strain HILLA-1 to degrade phenol and cresol in different concentrations

Results showed that *Acinetobacter baumannii* strain HILLA was capable of degrading phenol completely by 100% for all concentrations (25, 50, 75, 100, 200, 300, 400, 500 mg/L) after 48 hours of incubation, as shown in Table (3), with an increase in the rate of bacterial growth during the incubation period, as shown in Figure 4. Furthermore, the results revealed that the biodegradation ability of *Acinetobacter baumannii* strain HILLA to cresol for concentrations (25, 50, 75, 100, 200 ppm/L) was 100% while the concentrations (300, 400, 500 ppm/L) are 99.37%, 88.69%, and 94.73% respectively after 48 hrs as shown in Table (4) with an increase in the vital growth rate of isolate as in Figure 5.
Table 3: Biodegradation rates of Acinetobacter baumannii strain HILLA-1 for Phenol

<table>
<thead>
<tr>
<th>Start concentration of phenol mg/l</th>
<th>The remaining of phenol after degradation</th>
<th>% of Degradation after</th>
<th>Mean Degradation%/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 hrs.</td>
<td>24hrs.</td>
<td>48hrs.</td>
</tr>
<tr>
<td>500</td>
<td>376.11</td>
<td>121.66</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>362.77</td>
<td>88.33</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>221.66</td>
<td>42.77</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>131.66</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>98.88</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>70.22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>17.22</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4: Biodegradation rates of Acinetobacter baumannii strain HILLA-1 for Cresol

<table>
<thead>
<tr>
<th>Start concentration of cresol ppm/l</th>
<th>The remaining of cresol after degradation</th>
<th>% of Degradation after</th>
<th>Mean Degradation%/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 hrs.</td>
<td>24hrs.</td>
<td>48hrs.</td>
</tr>
<tr>
<td>500</td>
<td>479.66</td>
<td>74.11</td>
<td>26.33</td>
</tr>
<tr>
<td>400</td>
<td>391.88</td>
<td>45.22</td>
<td>45.22</td>
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<tr>
<td>300</td>
<td>271.88</td>
<td>33</td>
<td>1.88</td>
</tr>
<tr>
<td>200</td>
<td>183</td>
<td>38.55</td>
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<tr>
<td>100</td>
<td>96.33</td>
<td>47.44</td>
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<tr>
<td>75</td>
<td>73</td>
<td>29.66</td>
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</tr>
<tr>
<td>50</td>
<td>50</td>
<td>16.33</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>15.44</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Discussion

Petroleum compounds and its derivatives polluting the environment threaten human health and many other living organisms present in land and marine systems, so getting rid of pollution by oil vehicles is very important at various levels. Biological treatment is one of the most important techniques available in removing pollution as it is less dangerous than other types of treatments. It is a physical and chemical purification of the environment from pollutants by using microorganisms naturally present in such environments because of their ability...
to break down dangerous compounds into less toxic compounds.

**Conclusion**

We have selected and identified a local *Acinetobacterbaumannii* strain, which degrades phenol and cresol as the only carbon sources. Our strain showed a high efficiency in breaking down even high concentrations of phenol (500 mg/L) and cresol (500 ppm/L). Therefore, *Acinetobacterbaumannii* strain HILLA-1 may be taken into consideration as a helpful biotechnological means for the treatment of diverse environments contaminated with phenol or cresol.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved and all experiments were carried out in accordance with approved guidelines.

**References**


Evaluating Growth Charts Use among Children Less Than Five Years of Age at Primary Health Care Centers in Al-Hillah City

Muna Abdulwahab Khaleel
Prof., Nursing College / Al-Bayan University / Baghdad / Iraq

Abstract

The study was conducted at two primary health care centers in Al Hilla city which covered Al Gameyah primary health care center and Al Kawthar primary health care center for the purpose of evaluating the use of growth chart and to determine mothers understanding toward the meaning of the weight chart in terms of growth. All registered children aged 0-5 years who were living in areas served by the above two mentioned primary health centers were included. The following results may be drawn on the objectives of the study. The proportion of registered children who possessed a chart was very high (99.8%). The proportion of registered children who acquired a chart at birth was high too (99.6%). It is of interest to find that the date of birth of (99.6%) of children was registered and the weight at birth of (99.2%) of them was known too. It was found that only (90%) of study population had their weight charted on the curve of growth charts. The study showed that no children of both sexes were attending regularly to have their weight charted in most months of the year. The study generally depicted that the level of immunization of the children in study area was remarkable and high.

Keywords: Growth Charts, Children, Age, Primary Health Care Centers

Introduction

Growth and development of children has fundamental importance in a country’s overall progress. Growth monitoring is widely accepted and strongly supported by health professionals, and is a standard component of community pediatric services throughout the world. The WHO has recommended use of growth chart (GC) for close monitoring of children’s growth. However, the availability of this tool does not automatically translate to its use. Knowledge of its meaning, usefulness and acceptance by the health professionals who are directly in charge of childcare is necessary. Many conferences were held in the world to discuss the health of children in general with special emphasis on growth monitoring; this was voiced loudly in Alma-Ata, USSR, 1978. Since eighties till now researchers devoted much time and efforts to child health and emphasized that growth monitoring is an important technique for identifying individuals, groups, communities whose growth is not keeping up with the expected pattern. Many Pediatricians believe that the two of the most important things about a healthy child are that he should be growing and that he should be about the right weight for his age. Laraway and others reported that growth standards have been developed in most countries of Europe and North America by measuring normal healthy children both cross-section ally and also by following their growth longitudinally over a period of time. Ben-Joseph and others stated that in developing countries where failure of growth is a common phenomenon, the need for suitable growth charts has been acutely felt, but the lack of appropriate local standards and absence of well-planned longitudinal studies of children are major obstacles. World Health Organization (WHO) pointed out that, growth charts provided three channels of growth in weight based on internationally acceptable optimum and it was adopted by many different countries. Moreover, the WHO (1978) organized all efforts to promote the more widespread use of growth chart in primary health care and recommended a prototype of growth chart with guidelines for chart’s use in health services.
other concerned organizations have shown their deep concern on the benefits of using the growth chart and they stated that it can offer a simple and inexpensive means of monitoring child health and nutritional status and can be used by community health workers with very little instructions and supervision. Researchers have emphasized that, regular monitoring of weights of children on a weight chart is helpful for the promotion of optimum growth in children and also they stated that, such diagnostic, curative and promotive / preventive uses of the growth chart have needed clarification in order to achieve full acceptance of their use in clinical as well field conditions. This study was conducted for the purpose of evaluating the growth charts use and to estimate the proportion of mothers understand the meaning of the weight chart in terms of growth.

Methodology

All registered children aged (0-5) years who were living in areas served by Al Gameyah primary health care center and Al Kawthar primary health care center at the time of the study were included. Interviewing of (400) mothers was conducted at the above two mentioned primary health centers and a question was asked whether they understood the meaning of the weight chart in terms of growth. The data were gathered from growth charts and health records available at the above two mentioned primary health care centers. The data described by using simple statistical analysis such as: frequencies, percentages to assess the results of the study.

Results and Discussion

Table (1) Chart – Carrying Children

<table>
<thead>
<tr>
<th>Variables</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registered children aged 0-5 years</td>
<td>12380</td>
<td>100</td>
</tr>
<tr>
<td>Registered children with charts</td>
<td>12355</td>
<td>99.8</td>
</tr>
<tr>
<td>Registered children who acquired chart at birth</td>
<td>12330</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Table (2) Registered Children with Known Birth Dates and Birth Weight

<table>
<thead>
<tr>
<th>Variables</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registered children aged 0-5 years</td>
<td>12380</td>
<td>100</td>
</tr>
<tr>
<td>Registered children with known birth date</td>
<td>12330</td>
<td>99.6</td>
</tr>
<tr>
<td>Registered children with known birth weight</td>
<td>12280</td>
<td>99.2</td>
</tr>
<tr>
<td>Registered children whose weight were recorded on the curve of growth chart</td>
<td>11170</td>
<td>90.0</td>
</tr>
</tbody>
</table>

Table (3) Median Number of Months Out of Each Year in Which Weight Was Charted

<table>
<thead>
<tr>
<th>Year of Life</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Second</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Third</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Fourth</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fifth</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table (4) Approximate Total Visits per Child per Year to Primary Health Care Center

<table>
<thead>
<tr>
<th>Year of Life</th>
<th>Visit / Child</th>
<th>Visit / Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Second</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Third</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Fourth</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Fifth</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>101</td>
</tr>
</tbody>
</table>

Table (5) Percentage of immunization status from growth charts

<table>
<thead>
<tr>
<th>Immunization</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>100</td>
</tr>
<tr>
<td>Polio</td>
<td>100</td>
</tr>
<tr>
<td>DPT</td>
<td>97.5</td>
</tr>
<tr>
<td>Measles</td>
<td>97.9</td>
</tr>
<tr>
<td>MMR</td>
<td>98.9</td>
</tr>
</tbody>
</table>

Table No (1) shows that children who registered in both primary health care centers aged from (0-5) years were 12380. The proportion of children who possessed a chart was very high (99.8%). Here the mothers considered the chart as a sort of passport to health care, and they were proud to possess it. It is of interest to report that the children who acquired a chart at birth were very high too (99.6%).

From table (2) it can be seen that between the ages of (0-5), of 12380 children registered in both PHC centers, the date of birth of 12380 (99.6%) was known, and the weight at birth of 12380 (99.2%) of them was known too while they were recorded in growth charts as normal ranged from 2.5kg-3.5kg (90%) of them only. This information will be valuable to the child as he grows up, as well as being important in assessing his health. In a study of the relationship between birth weight and child growth in Guatemala over a period of 9 years (1965-1974), the study showed that infants born with deficient weight tend to remain in lower growth tracks whether the variable measured was weight, height or head and chest circumferences through the entire length of the study. But here in this study the problem is the absence of a suitable method of monitoring and recording the weight of children regularly on a simple growth char. That means recording is in adequate, and this is because of the fact that health workers have little or no training in the symbolism involved in such charts, therefore it is difficult in assessing child’s health.

The research was done to discover how often the weight of children was charted in each year of their life. From table (3) it is clear that no children of both sexes, boys and girls in primary health care centers were attending regularly, therefore their weight was not charted in most months of the year. From the researcher’s observation, it was found that most of their attendances
were consistent with the time of receiving their vaccines and when they were sick.

Studies conducted on the evaluation of the use of growth chart in in Northeast District of Delhi, India and in Isfahan found inconsistent results in that a high proportion of children both sexes were attending regularly enough to have their weights charted in most months of the year $^{17,18}$. This difference in the results may be because the majority of mothers were not advised about the importance of weight chart in terms of growth. The study also showed how frequently children attended the clinic. The attendances for all children of the studied population were analyzed. From table (4) it was clear that mothers brought their children to the primary health care centers during their first three years of life especially the second year, this might due to the fact that mothers attended the clinic for the purpose of vaccination, while the numbers of attendances dropped during the fourth and fifth years of the children life. Besides that, the table shows that mothers brought their girls more than boys, and this finding is contradicting with most of Iraqi women’s customs because male are always more protective than female. Therefore, this result could be due to small sample size studied which represented (1.6%) of study population only.

Estimation was made for the level of immunizations of children in the area, and the results are shown in table (5). The study generally indicated that the level of coverage in the study area is remarkable and high as compared to some other developing countries $^{14}$.

The high level of immunization in the area is partly due to many important points, first is the coordination and cooperation between health authorities and other organizations. Second is the experience gained by health staff from different aspects of preventive programs in encouraging women to bring their children. Similarly, the campaigns and the mass media has an important role contributed to the knowledge of the public about health and prevention of diseases by immunization. So by using these approaches it was possible to contact defaulters.

A question was asked for (400) mothers in the primary health centers during the time of the study whether they understood the meaning of the weight chart in terms of growth, and those involved concluded that a very small proportion (26%) only had some awareness and understanding.

Finally, the investigator recommended that the responsible authorities should follow the best use of growth charts in all primary health care centers, and conducting training courses for nurses and other health care providers who are working with growth charts units.

Financial Disclosure: There is no financial disclosure.

Conflicts of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Bayan University and all experiments were carried out in accordance with approved guidelines.

References


Complications of Kalazzer among Children in Hilla-city

Ibtesam Khalid Kamil
Assistant Prof., Al-Bayan University, College of Nursing, Pediatric Nursing, Iraq

Abstract

Objective: This study aimed to detect the types of complications of children suffering from kalazar; also to identify the reason of these complications.

Methodology: A descriptive study carried out from Feb15th to June 15th of the academic year 2017. The sample consisted (109) children from general maternal and children hospital in Hilla/Babylon. The study designed and data was collected using structural questionnaire which was prepared by consulting panel research expertise, also by assessment approach and interview was done with parents of children. The collected data were analyzed through descriptive statistics by frecuency and percentage.

Results: The results of the present study indicated that most of the children age were (13-18) yrs.(40.4%); male gender more involved (67%) and (27%) were from Mesaib city. The education level of parents were within secondary school, mothers (52%) fathers (43%). The income of affected children families involved in the study were (51%) poor, which isn’t enough to maintain healthy well being status and medical requirements. Complications revealed that children suffers from bleeding tendency (13.5%) sever animea were (20%) hepato spleenomegaly were (14.0%) , finally death occurs because of septicemia (37%).

Conclusion: Complications accurs because of poor and inappropriate medical diagnosis, also infective medical treatment during hospitalization and lake of medical follow up to children suffering from kalazar by the health team after hospital discharge.

Keywords: Complications, Kalazar, Children, Hilla-city

Introduction

Kalazar is a parasitic disease caused by the visceral leishmaniasis. This parasite lives in infected dogs transfer to sand flies to human, then humans can also transmit the parasite between each other through a blood transfusion or shared needles. Different species of the parasite cause each form, cutaneous leishmaniasis affects the skin and usually not serious; visceral leishmaniasis damage the interior organs and can be life-threatening; mucocutaneous leishmaniasis can lead to partial or complete destruction of the mucous membranes found in the nose, throat, and mouth 1. The parasite common in tropical and subtropical environments, affected region are often remote and unstable, with limited resources for treating this disease. According to the (WHO), environmental and climate factors heavily influences the spread of disease; 350 million people all over the world are consedered at risk, and 2 million of new cases occur yearly. (90%) of cases occur in Mediterrean; Central West Asia; Middle East; North and East Africa; South and Latin America 2. In Iraq kalazar disease increased from 5% of people with visceral leishmaniasis in (1999-2000) to 9% in (2016-2017) 3. Socioeconomic conditions; poverty is a determining factorfor the disease, malnutrition, illiteracy, large migrations caused urbanization emergency situations and changes; infections and people who have weakened immune systems are also at increased risk of this conditions 4. The incidence rate of kalazar is from (1-14) yrs. of age, the disease occurs from 2-8 months after being bitten by a sand fly; typically lead to fever, malaise, skin ulcer and dark ashen color, shivering, loss of weight, anemia, digestive disorder, dyspnea, edema in the lower limbs,
lymphadenopathy, jaundice and hepatosplenomegaly and some patients progress with heavy bleeding, finally sepsis and renal failure leads to death. For diagnosis, skin biopsy by scraping one of the ulcers to identify the parasite, also physical and blood exam is required. Treatment include medication that contains antimony (e.g. meglumine and sodium stibogluconate). Ulcers on the face that cause disfigurement may require plastic surgery. The potential complication include bleeding and weakened immune system which can be life threatening and fatal. Death occurs due to complications of the disease and not to the disease itself.

Methodology

Design: A descriptive study was conducted, purposive sample of (109) children were suffering from kalaazar in Hilla-city. Questionnaire has been used as a tool of data collection, for the period of Feb. 15/2017 to June 15/2017 and consist of socio-demographic data; pts complains from the disease types of complications and causes of death. Data were analyzed using the descriptive statistical data analysis which include (frequencies and percentage).

Results

Table 1. Distribution of Socio-Demographic characteristics of research samples

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 yrs.</td>
<td></td>
<td>5</td>
<td>4.6</td>
</tr>
<tr>
<td>3-5 =</td>
<td></td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>6-12 =</td>
<td></td>
<td>38</td>
<td>3.5</td>
</tr>
<tr>
<td>13-18 =</td>
<td></td>
<td>44</td>
<td>40.4</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>73</td>
<td>67</td>
</tr>
<tr>
<td><strong>Residency</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td></td>
<td>54</td>
<td>49</td>
</tr>
<tr>
<td>Rural</td>
<td></td>
<td>55</td>
<td>51</td>
</tr>
<tr>
<td><strong>Parent Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>Mother</td>
<td>28 - 26</td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td></td>
<td>57 - 52</td>
</tr>
<tr>
<td>College</td>
<td></td>
<td></td>
<td>24 - 22</td>
</tr>
<tr>
<td><strong>Economic Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enough</td>
<td></td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Just enough</td>
<td></td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Not enough</td>
<td></td>
<td>51</td>
<td>47</td>
</tr>
</tbody>
</table>

Table (1) shows the highest percentage of the sample at age ranged from (13-18 yrs.) and they are accounted (40.4%) male were more affected, they were accounted far (67%). The highest percentage of the sample was represented in urban (49%) and rural were (51%); The percentage of parent education was secondary school level (52%) among the mothers and (43%) among the fathers. Economic status, the highest percentage of the sample was not enough, they were accounted (51%) concerning the families income status.
Table 2. (Part-I) Complains of children during hospitalization

<table>
<thead>
<tr>
<th>Child problem</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>106</td>
<td>15.7</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>86</td>
<td>12.8</td>
</tr>
<tr>
<td>Anemia</td>
<td>109</td>
<td>16</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>77</td>
<td>11.5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>67</td>
<td>10</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>50</td>
<td>7.5</td>
</tr>
<tr>
<td>Bleeding Tendancy</td>
<td>88</td>
<td>13</td>
</tr>
<tr>
<td>Hepato-spleenomegaly</td>
<td>91</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Note: The child may have more than one problem.

Table (2) shows that bleeding tendancy accounted (13%); anemia (16%); and Hepato-spleenomegaly (13.5%).

Table 3. Period of time during child hospitalization

<table>
<thead>
<tr>
<th>Time</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 wks</td>
<td>78</td>
<td>71.5</td>
</tr>
<tr>
<td>2-3 wks</td>
<td>21</td>
<td>19.3</td>
</tr>
<tr>
<td>3-4 wks</td>
<td>10</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Table (3) shows that period of time during child hospitalization is between (1-2 wks.), which count (71.5%).

Table 4 Types of complication

<table>
<thead>
<tr>
<th>Complications</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe anemia</td>
<td>88</td>
<td>20</td>
</tr>
<tr>
<td>Bleeding</td>
<td>59</td>
<td>13.5</td>
</tr>
<tr>
<td>Jaundice</td>
<td>71</td>
<td>16</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>55</td>
<td>12.5</td>
</tr>
<tr>
<td>Nephritis</td>
<td>65</td>
<td>15</td>
</tr>
<tr>
<td>Hepato-spleenomegaly</td>
<td>62</td>
<td>14</td>
</tr>
<tr>
<td>Death</td>
<td>38</td>
<td>9</td>
</tr>
</tbody>
</table>

Note: children suffer from more than one complication.
Table (4) shows that (13.5%) of the sample suffers from bleeding especially epistaxis 4%, haematuria 5%, malaria 3%, ecchymosis (1.5%). Also 20% of cases shows anemia problems, jaundice (16%) .

Finally death is a result of these complications (9%) of the children in the sample died because of lower existance and immunity and as a result of weak preventive measurement.

Table 5 Prognosis of children during hospitalization

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete recovery</td>
<td>37</td>
<td>34</td>
</tr>
<tr>
<td>Weak prognosis</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>Child getting worse</td>
<td>38</td>
<td>35</td>
</tr>
</tbody>
</table>

According to table (5), children with kalazar shows that (34%) got well and recovered from the disease completely; (31%) had weak prognosis and slow response to the therapy. (35%) of children in the sample gets bad prognosis and their condition becomes worse due to the complication of the kalazar disease.

Table 6 Cause of death among the children in the research study

<table>
<thead>
<tr>
<th>Causes</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory failure</td>
<td>11</td>
<td>29</td>
</tr>
<tr>
<td>Renal failure</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>Septicemia</td>
<td>14</td>
<td>37</td>
</tr>
</tbody>
</table>

Table (6) shows the causes of death among the sample, (37%) because of septicemia, (34%) had died because of renal failure and finally (29%) had died because of respiratory failure.

**Discussion**

This research study had been reported that the highest percentage (40.4%) of the sample was at age group (13-18) yrs. as shown in table (1), this finding was consistent with study[3] done in Iraq, they found the relation of kalazar with this age group, also male gender shows (67%) more than female (33%) because the increase risk factor of exposure to the infection. Parent education shows that father had secondary school level while mothers counted (43%) this means, they got the education for understanding the nature of kalazar 2; in term of providing health maintenance to their children. The highest percentage of the sample residency was presented in rural area(51%); from (Mesaib 27%, Haswa field 24%); while 49% from urban part in Hilla city and Mahaweel region. Concerning the economic status of children families income were (47%) low and not enough that means they couldn’t tolerate the cost of medications which were needed for their sick children, besides the requirements of their living status and health issues 8. Children with kalazar in the study shows a complications because of bleeding tendency (13.5%); while (20%) of the sample suffer from anemia; (14%) from hepatosplenomegaly 9. Children stay (1-2 wks) mostly (71.5%) of them; this period of time is not enough for better prognosis 10,11. Only (34%) of the children got complete recovery while (35%) of them gets worse condition. The main complications are septicemia(16%), Nephritis (15%) and pneumonia (12.5%). These complications lead’s the children with kalazar to death. Also respiratory failure (29%); renal failure (34%); and (37%) because of septicemia. Children becomes resistant to treatment due to their weak prevention to disease and poor immunity system 12.

**Recommendations:**

1- Encourage more experimental studies and educational programs concerning kalazar disease in terms of planning and implementing high standard of medical and nursing therapy, through organizing scientific efforts by the Iraqi Ministry of Health and the Academic work within the Iraqi Universities for better control of the disease and personal protection in the community.

2- Provide safety methods and environmental management especially in rural area in order to reduce epidemiological problems, also to interrupt the transmission of the disease in the community and decrease the mortality rate.

3- Apply effective network of communication through Broadcasting; Social Media to increase public information concerning kalazar disease in terms of
prevention and early detection of sick children, and to provide and maintain healthy approach of medical treatment and nursing care within the society.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved and all experiments were carried out in accordance with approved guidelines.

References


Assessment of Diabetes Patient Adherence to Dietary Recommendation in Diabetics Center in Al-Najaf city

Iman Qasim Kteo Al-hussein 1, Zahraa Abdull Abbass Taher Al-Khafajy 1, Haider Mohammed Halooob AL-Abedi 1, Athraa Abbas Al-Zeyadi 2

1 M.Sc. Adults Nursing, Faculty of Nursing, University of Kufa, 2 M.Sc. Maternal and Newborn Health Nursing

Abstract

Objective: This study aimed to assess of diabetes patient adherence to dietary recommendation in Diabetics center in Al-Najaf city and to find out the relationship between the diabetic patient adherence to dietary recommendation and their demographic and clinical data. Methodology: Descriptive analytic design was carried out to assess of diabetics patient adherence to dietary recommendation in Al-Najaf City/ Health Directorate of Al-Najaf Al-Ashraf / Al-Sadder Medical City / Al-Najaf Center for Diabetes and Endocrine. The study was carried out from February 1st, 2018 up to March 28th, 2018. A non-probability (accidental) sampling technique was used consisting of (100) female and male those who visit Al-Najaf Center for Diabetes and Endocrine. Data collected through using of a well-designed questionnaire consist of three parts: Part I: Socio-demographic Characteristics, Part II: Clinical Data: consists of (6) items and Part 11: Patient adherence to dietary recommendation. This part of the questionnaire consists of (16) items.

Conclusion: It is concluded that the Majority of the stay sample have fair adherence to diabetics dietary recommendations.

Recommendation: Based on study conclusion, its need for a good relationship between the patient and health-care provider in order to provide more understanding and knowledge about the disease and its non-medical management and diabetic’s patients need dietary recommendations with means of education, such as published materials with regard to dietary regimen.

Keywords: Diabetes, Patient, Diabetics Center in Al-Najaf city

Introduction

Diabetes mellitus (DM) consist an enormous public health problem globally, associated with high morbidity and mortality 1. Normally, a certain amount of glucose circulates in the blood. The major sources of this glucose are absorption of ingested food in the gastrointestinal tract and formation of glucose by the liver from food substances. Diabetes mellitus (DM) is a chronic progressive metabolic disorder characterized by hyperglycemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin hormone 2. The exact mechanisms that lead to insulin resistance and impaired insulin secretion in type 2 diabetes are unknown, although genetic factors are thought to play a role. Regardless of the specific cause, the destruction of the beta cells results in decreased insulin production, unchecked glucose production by the liver and fasting beta cells cannot keep up with the increased demand for insulin, the glucose level rises and type 2 diabetes develops Despite the impaired insulin secretion that is characteristic of type 2 diabetes, there is enough insulin present to prevent the breakdown of fat and the accompanying production of ketone bodies 3. The treatment of diabetes should start with non-pharmacological therapies such as lifestyle interventions. Diet plays a major role in the therapeutic strategy to keep patients with diabetes in good glycemic control and prevent micro and macro vascular complications 4. According to the American Diabetes Association (ADA) and the European Association for the study of Diabetes (EASD) prescribed dietary recommendations for the
treatment of this disease since many years. Type 2 Diabetes is defined as chronic hyperglycemia resulting from either decreased insulin secretion, impaired insulin action or both in the absence of autoimmune destruction of the pancreatic beta cell. Classically, type 2 diabetes occurs in the older, obese patients in the setting of strong family histories of diabetes and in association with other components of the metabolic syndrome. Unhealthy practices and perception for diabetic patients can lead to increase the effect of the disease and complications which can be prevented. The diabetic complications such as nephropathy and retinopathy and neuropathy can affect the quality of life of the patients and can lead to a significant effect on patient’s quality of life and productivity. Healthy dietary habits and lifestyle modifications the cornerstones of type 2 diabetes prevention and management. Adherence to lifestyle modification recommendations lessens the disease burden and reduces the morbidity and mortality associated with type 2 diabetic complications.

Methodology

Design of the study: Descriptive analytic design was carried out to assess of diabetics patient adherence to dietary recommendation in Al-Najaf City/ Health Directorate of Al-Najaf Al-Ashraf / Al-Sadder Medical City / Al-Najaf Center for Diabetes and Endocrine.

Study objectives: This study aimed to assess of diabetics patient adherence to dietary recommendation in Diabetics center in Al-Najaf city and to find out the relationship between the diabetic patient adherence to dietary recommendation and their demographic and clinical

Duration of the study: The study was carried out from February 1st, 2018 up to March 28th, 2018.

Sample of the Study: A non-probability (accidental) sampling technique was used consisting of (100) female and male those who visit Al-Najaf Center for Diabetes and Endocrine. The Study Instrument: Data collected through using of a well-designed questionnaire consist of three parts: Part I: Socio-demographic Characteristics: This part consists of (7) items, including (gender, age, and marital status, level of education, occupational status, residency and socio-economic status) and Part II: Clinical Data: consists of (6) items, including (duration of disease since diagnosis, treatment, health education regarding self-care activities, complications, smoking and body mass index). Part III: Patient adherence to dietary recommendation. This part of the questionnaire consists of (16) items, the items include assessing patients’ adherence to dietary recommendation.

Ethical consideration: This is essential value and should be obtained before collecting the information, to respect the patient’s dignity and values. The investigator can achieve this permission from the moral committee at the Nursing Department in the faculty of Nursing / University of Kufa. Also another agreement from Al-Najaf Al-Ashraf Health Directorate / Al-Sadder medical city / Al-Najaf Center for Diabetes and Endocrine. In addition to above, the researcher told each participant that this is an involuntary work, and they can leave any time even the interview process uncompleted.

Questionnaire Validity: The questionnaire validity faces validity for the initial developed instrument which is specified through panel of (5) experts (4) Experts from faculty of Nursing/University of Kufa (with experience of > 5 yrs at their jobs field). Who were asked to review the instrument, and to inspect relevancy, clarity, and sufficiency of the questionnaire to measure the concept of interest. The data was collected through the applying of the developed questionnaire with aid of structured interview technique with the subjects as they are individually interviewed. The data collection process started from March 1st, 2018 to March, 20th, 2018.

Statistical Analysis: In this study, the data were analyzed by using of (SPSS) program version 19 (Statistical Package for Science Service), and the statistical package (Excel 2013).
### Result

Table (1) shows the Study Sample Demographic Data and their discussion

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Rating and intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td></td>
<td>46.0%</td>
</tr>
<tr>
<td>Male</td>
<td>54</td>
<td></td>
<td>54.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Age / years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>40.00 - 44.00</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>45.00 - 49.00</td>
<td>12</td>
<td></td>
<td>12.0%</td>
</tr>
<tr>
<td>50.00+</td>
<td>86</td>
<td></td>
<td>86.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>86</td>
<td></td>
<td>86.0%</td>
</tr>
<tr>
<td>Single</td>
<td>4</td>
<td></td>
<td>4.0%</td>
</tr>
<tr>
<td>Divorced</td>
<td>9</td>
<td></td>
<td>9.0%</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Levels of education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>31</td>
<td></td>
<td>31.0%</td>
</tr>
<tr>
<td>Able to read and write</td>
<td>16</td>
<td></td>
<td>16.0%</td>
</tr>
<tr>
<td>Primary school</td>
<td>20</td>
<td></td>
<td>20.0%</td>
</tr>
<tr>
<td>Intermediate school</td>
<td>14</td>
<td></td>
<td>14.0%</td>
</tr>
<tr>
<td>High school</td>
<td>8</td>
<td></td>
<td>8.0%</td>
</tr>
<tr>
<td>Institute and college</td>
<td>11</td>
<td></td>
<td>11.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Occupational status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>Government employee</td>
<td>12</td>
<td></td>
<td>12.0%</td>
</tr>
<tr>
<td>Retired</td>
<td>12</td>
<td></td>
<td>12.0%</td>
</tr>
<tr>
<td>Free job</td>
<td>19</td>
<td></td>
<td>19.0%</td>
</tr>
<tr>
<td>Housewife</td>
<td>45</td>
<td></td>
<td>45.0%</td>
</tr>
<tr>
<td>Jobless</td>
<td>11</td>
<td></td>
<td>11.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Economic status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>27</td>
<td></td>
<td>27.0%</td>
</tr>
<tr>
<td>Barely sufficient</td>
<td>47</td>
<td></td>
<td>47.0%</td>
</tr>
<tr>
<td>Insufficient</td>
<td>26</td>
<td></td>
<td>26.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td><strong>Residency</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>84</td>
<td></td>
<td>84.0%</td>
</tr>
<tr>
<td>Rural</td>
<td>16</td>
<td></td>
<td>16.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Freq= Frequency, %= Percent.

According the above table, the majority (54.0%) of sample were male, (86.0%) of patients were (50) years old and more. (86.0%) of patients were married. Concerning the level of education (31.0%) of sample was illiterate. (45.0%) of patient housewife. (47.0%) of sample economic status were barely sufficient. (84.0%) of patients live in urban area.

**Table (2) statistical analysis of diabetic patient’s clinical data and their discussion**

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Rating and intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of disease / years</td>
<td>1-3</td>
<td>23</td>
<td>23.0%</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>11</td>
<td>11.0%</td>
</tr>
<tr>
<td></td>
<td>7-9</td>
<td>10</td>
<td>10.0%</td>
</tr>
<tr>
<td></td>
<td>10 And More</td>
<td>56</td>
<td>56.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>Treatment</td>
<td>Oral hypoglycemia</td>
<td>34</td>
<td>34.0%</td>
</tr>
<tr>
<td></td>
<td>Injection</td>
<td>14</td>
<td>14.0%</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>3</td>
<td>3.0%</td>
</tr>
<tr>
<td></td>
<td>All of them</td>
<td>11</td>
<td>11.0%</td>
</tr>
<tr>
<td></td>
<td>Oral hypoglycemia &amp;</td>
<td>13</td>
<td>13.0%</td>
</tr>
<tr>
<td></td>
<td>Injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oral hypoglycemia &amp;</td>
<td>17</td>
<td>17.0%</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Injection &amp; Diet</td>
<td>8</td>
<td>8.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>Education regarding self-care</td>
<td>Yes</td>
<td>72</td>
<td>72.0%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>28</td>
<td>28.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>16</td>
<td>16.0%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>84</td>
<td>84.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>BMI</td>
<td>Obese</td>
<td>37</td>
<td>37.0%</td>
</tr>
<tr>
<td></td>
<td>Overweight</td>
<td>38</td>
<td>38.0%</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>25</td>
<td>25.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table (1) reveals that the majority (56.0%) of patient’s duration of disease were 10 years and more. The most of patients used tablets route to taking drugs (34.0%). About (72.0%) of patients received self-care education. (84.0%) of sample are not smoking. Around (38.0%) of patient were overweight.

**Table (3) overall assessment of diabetic patients’ adherence to dietary recommendations**

<table>
<thead>
<tr>
<th>Overall assessment for patients’ adherence</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good adherence</td>
<td>5</td>
<td>5.0%</td>
</tr>
<tr>
<td>Fair adherence</td>
<td>91</td>
<td>91.0%</td>
</tr>
<tr>
<td>Poor adherence</td>
<td>4</td>
<td>4.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table (3) show, that majority (91.0%) of sample with fair adherence to dietary recommendations

**Table (4) Relationship between the diabetic patients’ adherence to dietary recommendations and their demographic and clinical data**

<table>
<thead>
<tr>
<th>Demographic And Clinical Data</th>
<th>Chi-Square Value</th>
<th>Df</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.099</td>
<td>2</td>
<td>.952 NS</td>
</tr>
<tr>
<td>Age / years</td>
<td>6.412</td>
<td>6</td>
<td>.379 NS</td>
</tr>
<tr>
<td>Marital Status</td>
<td>8.045</td>
<td>6</td>
<td>.235 NS</td>
</tr>
<tr>
<td>Levels of education</td>
<td>16.241</td>
<td>10</td>
<td>.093 NS</td>
</tr>
<tr>
<td>Occupation</td>
<td>24.608</td>
<td>10</td>
<td>.006 HS</td>
</tr>
<tr>
<td>Economic Status</td>
<td>0.469</td>
<td>4</td>
<td>.976 NS</td>
</tr>
<tr>
<td>Residency</td>
<td>0.326</td>
<td>2</td>
<td>.849 NS</td>
</tr>
<tr>
<td>Duration Of Disease</td>
<td>4.672</td>
<td>6</td>
<td>.587 NS</td>
</tr>
<tr>
<td>Treatment</td>
<td>32.755</td>
<td>14</td>
<td>.003 HS</td>
</tr>
<tr>
<td>Education</td>
<td>3.835</td>
<td>2</td>
<td>.147 NS</td>
</tr>
<tr>
<td>Complications</td>
<td>67.251</td>
<td>46</td>
<td>.022 S</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.326</td>
<td>2</td>
<td>.849 NS</td>
</tr>
<tr>
<td>BMI</td>
<td>2.963</td>
<td>4</td>
<td>.564 NS</td>
</tr>
</tbody>
</table>
Related to table (4) there were good relationships between the patient (occupation, type of treatment and complications) with their adherence to dietary recommendations.

Discussion

According to (Table 1) in the results, the study shows that the entire study sample (54%) are male. The finding is consistent with results of [9] which mentioned in their study result that majority of the study subject were male. Regarding their age, the majority (86%) of the research samples are at age group of (50) yrs and more. This result is in agreement with other study [10] in their study found that a third of the participants, (26.9%) aged 50 to 59 years. Concerning the marital status the majority (86%) of the study subjects are married. This finding reinforced with the study result of [11] which mentioned majority of the study sample were married.

In related to the level of education the most of the study samples (31%) are illiterate while regarding the diabetic patient occupational status, about (45.0%) are house wife. These results matching with the study result of [8]. Concerning socio-economic status, about half of the sample (47%) reveals their economic status that is barely sufficient, while concerning the residency the study result show that highest percentage (84%) of the study sample are lived in urban area. These result reinforced with the study of [2] which mentioned that 80% of people with diabetes live in low- and middle-income countries.

Concerning duration of disease in the (table 2), the higher percentage (56%) is for those who are suffering from the diabetic for period from 10 years and more. This result comes along with the findings of other study which is carried out by [12].

Regarding the type of treatment, the results of the study display that the highest percentage (34%) of the study subjects are with oral hypoglycemia treatment. This result matching with the study result of [9] which mentioned the majority of participants were taking oral hypoglycemic medications. The present study shows that in regards to the education related to self-care, the most of the study subject (72%) are not received education related to self-care. This finding may related to the lack of nursing education and instruction to the patient about the dietary and exercise recommendation for the management of the diabetic disease and to reduce the complications related to disease. Related to the smoking, the majority of the study samples (84%) are not smoker. These findings matching with the study done by [13] who represents the majority of their study sample were not smoker. In regard to the study subject body mass index, the most of the study subject (38%) are overweight. These result agreements with the study finding done by [14] that represent the majority of their sample were overweight. Concerning the complications related to disease, the highest percentage of the study sample suffers from Neuropathy & Eye problems.

According to the above (table 3) in the result, the overall assessment of diabetic’s patient adherence to dietary recommendation is fair. This outcome is reinforced by a study done by [12] who concluded in their study results that the level of adherence to dietary recommendations in the study group was moderate.

Table (4) in the result shows that there are high significant relationship between two items (occupation and treatment ) and patient’s adherence to dietary recommendation at p-value less than 0.05, in addition there were significant relationship between the complication related to the disease and patients adherence to dietary recommendation at p-value less than 0.05. While there are non-significant relationship between the other items of diabetic patients demographic and clinical data and their adherence to dietary recommendation at p-value more than 0.05.

Conclusions

1- It is concluded that the most of the research sample are male and their dominant age are within 50 years and more, the most of them are illiterate. It was summaries that the most of the study sample taking oral-hypoglycemia medication, most of them not received education related to the disease and their dietary recommendation and in regard to their BMI it’s concluded that most of them are overweight. It is concluded that the Majority of the stay sample have fair adherence to diabetics dietary recommendations. It is concluded that there are significant relationship between the patient (type of treatment, occupation and complication related to disease) and their adherence to dietary recommendations.
Recommendations:

1. Based on study conclusion, its need for a good relationship between the patient and health-care provider in order to provide more understanding and knowledge about the disease and its non-medical management. Encourage the diabetic patients who visit the diabetic center to follow the healthy dietary recommendations. Diabetic’s patients need dietary recommendations with means of education, such as published materials with regard to dietary regimen. It is recommended to design proper booklet by the diabetic’s center to guide the visitors about the non-medical management of diabetic’s disease.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Dentistry, University of Babylon, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

References

5. Guidelines for Clinical Care Ambulatory, Management of Type 2 Diabetes Mellitus, UMHS, 2017.
Impact of Adolescents’ Life Style and Reasons of Skipping Essential Meals upon their BMI at Secondary Schools in Baghdad City

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1Lecturer- Collage of Nursing- Al-Bayan University, 2 Prof., The Dean Collage of Nursing, University of Baghdad

Abstract

Background: Excessive harmful and unhealthy weight-control behaviors have potential serious consequences due to impact on their life style. Specifically dieting is considered a major risk factor for eating disorders. Unhealthy dieting can also decrease intake of energy and essential nutrients.

Objective: The study aimed to identify the adolescents’ lifestyle, and reasons of skipping essential meals and find out the relationship between adolescents’ lifestyle and reasons of skipping essential meals and their demographic data (gender and Body Mass Index).

Methodology: A descriptive study design was conducted on impact of lifestyle and reasons of skipping essential meals upon adolescents’ Body Mass Index in secondary schools at Baghdad city, starting from 20th of April 2013 to the end of October 2014. Non-probability (purposive) sample of 1254 adolescents were chosen from secondary schools of both sides of Al-Karkh and Al-Russafa sectors. Data was collected through a specially constructed questionnaire format include (20) items multiple choice questions. The validity of the questionnaire was determined through a panel of experts related to the field of the study, and the reliability through a pilot study. The data were analyzed through the application of descriptive statistical analysis frequency, & percentages, and inferential statistical analysis, chi-square, are used.

Results: Regarding the gender, finding indicates that males and females (48.7, and 51.3%) respectively, were approximately equal, and the result study samples find more than one forth BMI were almost equal in under and normal weight percentile (27.8%, and 27.8%) respectively, they spent mostly time (5 hours and more) in watching TV, and use the computer for playing games, and sometimes eat during watching TV or playing computer games. Adolescents’ gender has highly significant association with times sharing with physical activity per week but the other items have no significant association. Adolescents’ Body Mass Index has highly significant association with their lifestyle. Adolescents’ gender has highly significant association with the reasons of skipping breakfast meal and the adolescents’ Body Mass Index have no significant association with the reasons of skipping meals

Key wards: Lifestyle, skipping essential meals, Body Mass Index.

Introduction

Adolescents’ media exposure is one of important factors for accessibility of fast food and snack food through advertisement messages. This factor is representing external environmental factors from adolescent development theory and can shape youth’s eating behavior according to specific situation differentials. Logically, number of media in the family can be addressed in eating habits clearly as when youth had exposed too many kinds of media frequently. Youth can follow and imitate according to famous stars from media and advertisements. In addition, when youth were accessed to many shops selling variety of fast food and snacks, they will be tried to buy and got these with reasons of modernization as well as busy schedules of businesses. (1) In regard to mass media, teenage group is the main target group of consumer product that is all luxury goods or malnutrition food such as high
sugar, high fat, high calorie, high sodium food, snack in attractive package candies, or chewing gum and so on. Especially teenagers always regard consumption as fashion by following frequent commercial advertisement such as televisions, radios, magazines, movies, and imitation from celebrities or favorite persons such as movie stars, singers, actors, actresses, sportsmen as well as copying friends in the group. Moreover, a number of studies in other countries such as Australia and the United Kingdom have found that television advertising to children for high sugar and high fat foods is prevalent \(^{(1)}\). In Thailand, advertising of all forms, but particularly television advertising, encourages children to eat unhealthy foods. Many children spend several hours a day in front of the television and computer play games. It can perhaps be said that families and advertisements together create sugar addiction among children. Some companies even promote their products for children inside school and other free samples to create brand loyalty. Children who watch a lot of television are less physically fit and more likely to eat high fat and high energy snack foods and some candies, because television takes time away from play and exercise activities. \(^{(2, 3)}\) Commercials for healthy food make up only 4% of the food advertisements shown during children’s viewing time. The number of hours of television viewing also corresponds with an increased relative risk of higher cholesterol levels in children. Television and computer using can also contribute to eating disorders in teenage girls, who may emulate the thin role models seen on television. Eating meals while watching television should be discouraged because it may lead to less meaningful communication and, arguably, poorer eating habits. \(^{(4, 5)}\)

Parents may also influence not only on adolescents but also on their child’s behaviors, and ultimately weight, through the social-emotional climate in the home, particularly given evidence suggesting that overweight in adolescence is associated with greater family conflict less family cohesion. The family social-emotional climate in the context of pediatric health studies includes variables, such as level of cohesion, adaptability and connectedness, as well as family meal environment. There has been limited focus on associations between family climate and specific weight-control behaviors and body image among adolescents, it has been suggested in some reasons is significantly associated with disordered eating and weight-control behaviors among adolescents, with lower family cohesion and adaptability predictive of binge eating, and use of extreme weight loss behaviors, such as vomiting and crash dieting among adolescent females. \(^{(6)}\)

Family connectedness, prioritizing of family meals, and a positive family mealtime environment have been found to be more inversely associated with unhealthy weight-control behaviors (UWCB), self-esteem, and body satisfaction among at-risk for overweight and youth overweight, suggesting that the family social-emotional climate dose not only affect on behavior of adolescents, but also on their cognitions. Family meal structure (e.g., fewer family meals), and more than familial expression of weight concerns. More evidence is needed to assess the effect of parent and family variables on weight-control behaviors, overeating and body image, particularly in an overweight population of adolescents, given that this population appears to be at greater risk for use of extreme weight-control behaviors and binge eating. \(^{(7, 8)}\)

There are weight control strategies which may include healthy behaviors such as moderate dieting and exercise, and can provide potential health benefits to adolescents to unhealthy eating practices including skipping meals, fasting, restricting intake of certain foods, and chronic dieting behaviors as well as potentially harmful behaviors such as self-induced vomiting laxatives, and diet pills, and adolescents smokers. \(^{(9)}\)

Excessive harmful and unhealthy weight-control behaviors have potential serious consequences due to their impact on physical emotional and psychosocial health. Specifically dieting is considered a major risk factor for eating disorders. Unhealthy dieting can also decrease intake of energy and essential nutrients and may be associated with a variety of symptoms including fatigue anxiety, and constipation and irregular menstrual cycles among girls. \(^{(10)}\)

**Methodology**

A descriptive study design was conducted on impact of lifestyle and reasons of skipping meals upon adolescents’ Body Mass Index in secondary schools at Baghdad city, starting from 20\(^{th}\) of April 2013 to the end of October 2014. Non-probability (purposive) sample of
1254 adolescents were chosen from secondary schools of both sides of Al-Karkh and Al-Russafa sectors. Data was collected through a specially constructed questionnaire format include (20) items multiple choice questions. The validity of the questionnaire was determined through a panel of experts related to the field of the study, and the reliability through a pilot study. The data were analyzed through the application of descriptive statistical analysis frequency, & percentages, and inferential statistical analysis, chi-square, are used.

The Results

Table (1) Distribution of the Study Sample by their Personal Information

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>611</td>
<td>48.7</td>
</tr>
<tr>
<td>Female</td>
<td>643</td>
<td>51.3</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under weight</td>
<td>349</td>
<td>27.8</td>
</tr>
<tr>
<td>Normal</td>
<td>348</td>
<td>27.8</td>
</tr>
<tr>
<td>Over Weight</td>
<td>293</td>
<td>23.4</td>
</tr>
<tr>
<td>Obese</td>
<td>241</td>
<td>19.2</td>
</tr>
<tr>
<td>Morbidity Obese</td>
<td>23</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*No. = number, % = percentage

This table shows that more than half (51.3%) of adolescents were female, nearly one third at (27.8%) of them under and normal weight of the students’ BMI

Table (2) Distribution of the Study Sample by their Responses to the Adolescents’ Life Style

<table>
<thead>
<tr>
<th>Adolescents’ Life Style</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watching TV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hours and above</td>
<td>451</td>
<td>35.9</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>163</td>
<td>13.0</td>
</tr>
<tr>
<td>1-2 hours</td>
<td>319</td>
<td>25.5</td>
</tr>
<tr>
<td>seldom</td>
<td>321</td>
<td>25.6</td>
</tr>
<tr>
<td>Eating food when watching TV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>320</td>
<td>25.5</td>
</tr>
<tr>
<td>sometimes</td>
<td>861</td>
<td>68.7</td>
</tr>
<tr>
<td>no never</td>
<td>73</td>
<td>5.8</td>
</tr>
<tr>
<td>Eating during doing homework</td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>54</td>
<td>4.3</td>
</tr>
<tr>
<td>sometimes</td>
<td>699</td>
<td>55.7</td>
</tr>
<tr>
<td>no never</td>
<td>501</td>
<td>40.0</td>
</tr>
<tr>
<td>Time for using network</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hours and above</td>
<td>350</td>
<td>27.9</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>157</td>
<td>12.5</td>
</tr>
<tr>
<td>1-2 hour</td>
<td>256</td>
<td>20.4</td>
</tr>
<tr>
<td>no never</td>
<td>491</td>
<td>39.2</td>
</tr>
<tr>
<td>eating when using the computer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>56</td>
<td>4.5</td>
</tr>
<tr>
<td>sometimes</td>
<td>422</td>
<td>33.6</td>
</tr>
<tr>
<td>No never</td>
<td>776</td>
<td>61.9</td>
</tr>
<tr>
<td>Times sharing with physical activity per week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 and above</td>
<td>100</td>
<td>8.0</td>
</tr>
<tr>
<td>5-6 times</td>
<td>144</td>
<td>11.5</td>
</tr>
<tr>
<td>3-4 times</td>
<td>87</td>
<td>6.9</td>
</tr>
<tr>
<td>1-2 times</td>
<td>885</td>
<td>70.6</td>
</tr>
<tr>
<td>no one</td>
<td>38</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*No. = number, % = percentage
This table shows more than one third (35.9%) of adolescents’ watch TV for 5 hours and above, two thirds (68.7%) sometimes eat foods when watching TV, about half percent (55.7%) sometimes eat during their homework doing, more than one fourth (27.9%) using network 5 hours and above when they more than one third (39.2%) never using network, one third (33.6%) sometimes eat when using computer while more than half (61.9%) never using it when they eat, most of the sample (70.6%) one to two times share in physical activity per week.

Table (3): Distribution of the Study Sample by their Reasons of Skipping Meals

<table>
<thead>
<tr>
<th>Reasons of Skipping Breakfast Meal</th>
<th>Always</th>
<th>Sometimes</th>
<th>Seldom</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>No appetite at morning</td>
<td>574</td>
<td>45.7</td>
<td>383</td>
<td>30.6</td>
</tr>
<tr>
<td>Difficulty to prepare breakfast or no time to eat</td>
<td>366</td>
<td>29.1</td>
<td>328</td>
<td>26.2</td>
</tr>
<tr>
<td>Saw myself overweight</td>
<td>138</td>
<td>14.6</td>
<td>270</td>
<td>21.5</td>
</tr>
<tr>
<td>Someone preparing the meal at morning</td>
<td>44</td>
<td>3.5</td>
<td>139</td>
<td>11.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reasons of Skipping Lunch and Dinner Meal</th>
<th>Always</th>
<th>Sometimes</th>
<th>Seldom</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Eating favorite food</td>
<td>95</td>
<td>7.6</td>
<td>318</td>
<td>25.3</td>
</tr>
<tr>
<td>Have a feud or conflict with anyone of my family</td>
<td>106</td>
<td>8.5</td>
<td>163</td>
<td>13.0</td>
</tr>
<tr>
<td>Eating snacks from out when I go to school</td>
<td>333</td>
<td>26.5</td>
<td>309</td>
<td>24.7</td>
</tr>
<tr>
<td>Fasting to keep my weight</td>
<td>84</td>
<td>6.7</td>
<td>260</td>
<td>20.7</td>
</tr>
<tr>
<td>Having lunch at late time</td>
<td>229</td>
<td>18.3</td>
<td>490</td>
<td>39.0</td>
</tr>
<tr>
<td>Forget to eat when I play computer games or watch TV</td>
<td>252</td>
<td>20.1</td>
<td>311</td>
<td>24.8</td>
</tr>
<tr>
<td>Eating my dinner at midnight</td>
<td>150</td>
<td>12.0</td>
<td>394</td>
<td>31.4</td>
</tr>
<tr>
<td>Sleeping early without eat any food</td>
<td>88</td>
<td>7.0</td>
<td>217</td>
<td>17.3</td>
</tr>
</tbody>
</table>

No. = number, % = percentage
This table shows that near to half (45.7%, and 29.1%) always adolescents’ have no appetite at morning and difficulty to prepare breakfast or no time to eat, more than one fourth with nearly on fourth (21.5%) sometimes saw myself overweight, two third (74.5%) seldom no one preparing the meal at morning. More than one third (25.3%, and 24.6%) there was sometimes and seldom eating favorite food, (63.6%) adolescents’ have no a feud with the family, more than one forth (26.5%) always eat snacks from out when they go to school, two third (62.2%) did not fast to keep their weight, (39.0%) sometimes late eat the lunch, and more than one third and half (42.1%, and 56.1%) did not forget to eat when they play computer games or watch TV and sleep early without eat any food, and (31.4%, and 16.5%) sometimes and seldom eat the dinner at midnight.

Table (4) Association between Adolescents’ lifestyle and their Gander

<table>
<thead>
<tr>
<th>Adolescents’ lifestyle</th>
<th>Gender</th>
<th>Total</th>
<th>X2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hours and above</td>
<td>218</td>
<td>233</td>
<td>451</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>80</td>
<td>83</td>
<td>163</td>
</tr>
<tr>
<td>1-2 hours</td>
<td>157</td>
<td>162</td>
<td>319</td>
</tr>
<tr>
<td>seldom</td>
<td>156</td>
<td>165</td>
<td>321</td>
</tr>
<tr>
<td>X2 = .995 Sig. = .995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating Food when watching TV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>156</td>
<td>164</td>
<td>320</td>
</tr>
<tr>
<td>sometimes</td>
<td>420</td>
<td>441</td>
<td>861</td>
</tr>
<tr>
<td>no never</td>
<td>35</td>
<td>38</td>
<td>73</td>
</tr>
<tr>
<td>X2 = .999 Sig. = .999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating during school duty</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>27</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>sometimes</td>
<td>339</td>
<td>359</td>
<td>698</td>
</tr>
<tr>
<td>no never</td>
<td>245</td>
<td>257</td>
<td>502</td>
</tr>
<tr>
<td>X2 = .979 Sig. = .979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET (time for using network)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hours and above</td>
<td>171</td>
<td>179</td>
<td>350</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>77</td>
<td>80</td>
<td>157</td>
</tr>
<tr>
<td>1-2 hour</td>
<td>126</td>
<td>130</td>
<td>256</td>
</tr>
<tr>
<td>no never</td>
<td>237</td>
<td>254</td>
<td>491</td>
</tr>
<tr>
<td>X2 = .999 Sig. = .999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating when using computer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>28</td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td>sometimes</td>
<td>208</td>
<td>214</td>
<td>422</td>
</tr>
<tr>
<td>No never</td>
<td>375</td>
<td>401</td>
<td>776</td>
</tr>
<tr>
<td>X2 = .997 Sig. = .997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Times sharing with physical activity per week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 and above</td>
<td>97</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>5-6 times</td>
<td>115</td>
<td>30</td>
<td>145</td>
</tr>
<tr>
<td>3-4 times</td>
<td>62</td>
<td>25</td>
<td>87</td>
</tr>
<tr>
<td>1-2 times</td>
<td>315</td>
<td>569</td>
<td>884</td>
</tr>
<tr>
<td>no one</td>
<td>22</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>X2 = .000 Sig. = .000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Χ² = Chi- square, sig. = significant, p-value ≤ 0.05
This table shows that adolescents’ gender has highly significant association with times sharing with physical activity per week but the other items have no significant association.

### Table (5) Association between Adolescents’ lifestyle and their BMI

<table>
<thead>
<tr>
<th>Adolescents’ lifestyle</th>
<th>Body Mass Index</th>
<th>Total</th>
<th>X2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Under Weight</td>
<td>Normal Weight</td>
<td>Over Weight</td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hours and above</td>
<td>126</td>
<td>129</td>
<td>81</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>60</td>
<td>39</td>
<td>36</td>
</tr>
<tr>
<td>1-2 hours</td>
<td>86</td>
<td>93</td>
<td>80</td>
</tr>
<tr>
<td>Seldom</td>
<td>77</td>
<td>87</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watching Food when Watching TV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>110</td>
<td>71</td>
<td>80</td>
</tr>
<tr>
<td>sometimes</td>
<td>218</td>
<td>266</td>
<td>195</td>
</tr>
<tr>
<td>No never</td>
<td>21</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating during School Duty</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>4</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>sometimes</td>
<td>192</td>
<td>189</td>
<td>161</td>
</tr>
<tr>
<td>No never</td>
<td>153</td>
<td>144</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NET (time for using network)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 hours and above</td>
<td>101</td>
<td>109</td>
<td>70</td>
</tr>
<tr>
<td>3-4 hours</td>
<td>15</td>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>1-2 hour</td>
<td>54</td>
<td>83</td>
<td>60</td>
</tr>
<tr>
<td>No never</td>
<td>179</td>
<td>106</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eating when using Computer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>always</td>
<td>14</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>sometimes</td>
<td>102</td>
<td>152</td>
<td>90</td>
</tr>
<tr>
<td>No never</td>
<td>233</td>
<td>184</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Times Sharing with Physical Activity per week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 and above</td>
<td>24</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>5-6 times</td>
<td>29</td>
<td>55</td>
<td>28</td>
</tr>
<tr>
<td>3-4 times</td>
<td>37</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>1-2 times</td>
<td>238</td>
<td>233</td>
<td>227</td>
</tr>
<tr>
<td>No one</td>
<td>21</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

$X^2 = \text{Chi-square}, \text{ sig.} = \text{significant, p-value} \leq 0.05$

This table shows that adolescents’ Body Mass Index has highly significant association with their lifestyle at p-value ≤ 0.05.
Table (6) the Association between Reasons of Skipping Meals of the Study Sample and their Gender

<table>
<thead>
<tr>
<th>Reasons of Skipping Meals</th>
<th>Gender</th>
<th>Total</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Reasons of skipping breakfast meal</td>
<td></td>
<td></td>
<td>364</td>
</tr>
<tr>
<td>Healthy</td>
<td>109</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>Unhealthy</td>
<td>502</td>
<td>388</td>
<td>890</td>
</tr>
<tr>
<td>Total</td>
<td>611</td>
<td>643</td>
<td>1254</td>
</tr>
<tr>
<td>Reasons of skipping lunch and dinner meal</td>
<td></td>
<td></td>
<td>565</td>
</tr>
<tr>
<td>Healthy</td>
<td>264</td>
<td>301</td>
<td></td>
</tr>
<tr>
<td>Unhealthy</td>
<td>347</td>
<td>342</td>
<td>688</td>
</tr>
<tr>
<td>Total</td>
<td>611</td>
<td>643</td>
<td>1254</td>
</tr>
</tbody>
</table>

χ² = Chi-square, sig. = significant, p-value ≤ 0.05

This table shows that adolescents’ gender has highly significant association with the reasons of skipping breakfast meal but the other item have no significant association.

**Discussion**

In the present study as shown in table (1) refers to statistically distribution of the observed frequencies, percentages of all studied sample demographical characteristics variables. Regarding to the gender, the finding indicates that males and females (48.7, and 51.3%) respectively, were approximately equal. This study was nearly agrees with Romanian high schools study sample (43.1%, and 56.9%) for male and female respectively. The result study samples find that more than one forth BMI were almost equal in under and normal weight percentile (27.8%, and 27.8%) respectively, that indicate they did not have good nutrients for developing their physically and psychologically performance and this result supported with Romanian study because most of the study is indicated about under and normal weight for both genders (16.5% and 73.4%) respectively. (11)

New Mexico middle school students recently surveyed, 20% reported watching two hours of TV on an average school day, and 16% watch five or more hours per day. Half of all high school students watch from one to three hours of TV on an average day, while 20% watch four or more hours. (12) Regarding to adolescents’ lifestyle in table (2and 3) this result agrees with the finding of Romanian study most of the sample spent mostly times eating when watching TV and computer games. (1)

Adolescents’ gender has highly significant association with times sharing with physical activity per week but the other items have no significant association shows that in table (4), this results agrees with adolescents Damascus, Syria study . (13)

Adolescents’ Body Mass Index has highly significant association with their lifestyle shows that in table (5), this results was disagrees with adolescents Saudi study shown that no significant association BMI with Saudi adolescents’ lifestyles . (14)

In tables (6)(7) there are many reasons lead adolescents always and sometimes skipping the breakfast meal like, no appetite at early morning when they want to go to school, or no time to prepare their meal or no anyone to prepare it, and some of them self-esteem overweight. While the study samples’ reasons of
skipping lunch and dinner meal by mostly not eating the favorite food, or they have a conflict with their family, always eating snacks when they go to school, did not fasting to keep their weight, or because of the schools time the adolescents ate their lunch late, sometimes eating more at watching TV and playing by computer games, and eating at midnight, while they did not sleep after eating at night. This result agrees with the Turkish study. (15)

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Al-Bayan University and all experiments were carried out in accordance with approved guidelines.

**References**

Effectiveness of Some Relaxation Exercises Use by Nurse in Assessment of Vital Signs

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Abstract

Objectives: The objective of this study was to assess the short term effect of relaxation exercise on vital signs measured at baseline.

Method: A quasi-experimental design with pre and post-intervention with a group control method. Sixty subjects, divided to study and coronal group measurement of vital signs (Temperature, pulse, respiration and blood pressure) done by researchers, by using a calibrated sphygmomanometer, mercury thermometer, and done of four relaxation exercises include (deep breathing, leg relaxation exercise, arm relaxation exercise, and face muscles relaxation exercise).

Results: The findings of research appear there is a significant difference between the study and control sample in vital signs after applied relaxation techniques, and This table reveals the statistical significance and high significance between study and control study for the physical parameter (vital signs) after applied the relaxation technique.

Conclusions: The currents study concludes, some relaxation exercises such as (deep breathing, leg relaxation exercise, arm relaxation exercise, and face muscle relaxation exercise) after application for a study group, make a slight alteration in vital signs. And there is no significant association among the control group. The authors recommend the training of the office employees about some relaxation exercises.

Keywords: Effect, Nurse, Relaxation Exercise, Vital Signs, Measurement.

Introduction

The application of relaxation exercise in reduce anxiety and pain relief mentioned in several works of literature and have well resulted in reducing pain, anxiety, and lowering vital signs 1-3. Regarding surgical intervention, were the highest levels of anxiety and fear, the nurse can play a pivotal role in lowering a patient’s vital signs related to anxiety from surgery 4. Also, Nurses play a role in the adaptive response and rise patient experience to emotional problems before the surgical procedures which affect blood pressure, pulse, and respiratory rate to be near baseline 2. On the other hand, patients with maladaptive responses to pain and anxiety can play a negative role not only in need of sedative or opioids drugs but in the length of hospital stay 2,5. According to literature, there are several unpleasant feelings and discomfort associated with the stressed and anxious patient include muscles tight up such as headache, backache, neck and shoulder pain, instability of breathing, panic, palpitation, tremor, numbness in the hand and the face, as a result, relaxation is the most effective way of how to control the physical and feeling. There are many hormones such as cortisol, glucagon, adrenaline, epinephrine, and norepinephrine are connected with stress as well as there are many
physiological indicators including blood pressure, heart rate, respiratory rate, and body temperature. According to Kim et al. (2016) progressive muscle relaxation exercise creates a positive impression, to reduce the level of stress that evidenced by low blood pressure, slow breathing and decrease heart rate. The ability of people to be relaxed is required to learn, it is skilled more than natural ability. Moreover, relaxation exercise is safe, cost-effective, availability, and easy to nurse to teach the patients in multi health care setting. The current study measures the effects of some relaxation exercise in making a slight alteration in vital signs for persons in the workplace, the use of the inexpensive method is available can make positive effects regarding physiological body changes and can increase productivity.

Methodology

Research Hypothesis: the author hypothesized the most study subjects, have slight changes in vital signs after relaxation exercise practice.

Design: Descriptive design study was conducted using a questionnaire to collect data.

Setting and Data Collection: Twenty subjects in the college of nursing university of Al-Muthanna who met the inclusion criteria were vital signs measured and intervention of relaxation exercise and after that vital signs were measured.

Ethical Approval: This study was approved and permitted by the ethical committee in the college of the Nursing University of Al-Muthanna. The purpose of the study was explained to the participants. Informed consent was reviewed and permitted by the ethical committee in the college of the Nursing University of Al-Muthanna.

Study sample: the researchers applied this research on (60) employer office, Instructor, and worker are the population in this study. The target population that provided the sample data is a group of subjects who met the sampling criteria. All subjects with different educational levels were included in this study.

Instrument: study instrument includes

This tool consisted of two parts:

Part I: Subjects demographics characteristics include: age, gender, level of education, occupation.

Part II: Vital signs measurement before and after relaxation exercise intervention.

Data Analysis: The statistics done by the Statistical Package for the Social Sciences (SPSS) version 22 software was used to perform the statistical analysis. By use frequency, percentage, mean, slandered deviation, and dependent and independent t-test.

Findings

The current findings of 60 clients who participate in this study, were their mean age of the study group 37.90, and the control group was 38.06 mean of age and the majority (30%) was 29-35 years old. Regarding gender distribution, the majority of the study sample was female in the study group (66.7%) and the control group(73%). The quarter 26.7% of the study group was a bachelor’s degree and one-third (33.3%) of the control group also bachelor’s degree. According to marital status, the most (86.6%) of the study group was married in the same manner the most (93.3%) of the control group also married. Finally, according to the residence, the majority of 66% of the study group was urban, and near two-third (73%) of the control group also residence in the urban area see table (1).

In comparison between study and control groups of vital signs measurement, the study group systolic blood pressure SBP was 114.63 mean of scores and the control group was record 118.63 mean of score SBP readings and have a significant association between study and control groups(t=-2.379,p = 0.020). The diastolic blood pressure DBP reading for the study group recorded 62.13 mean of the score, and 65.00 mean of scores for the control group, and The single most marked observation to emerge from the data comparison was the significant association between study and control group (t=-3.627,p = 0.001). The findings also show the body temperature record 37.06 mean of the score for the study group, and body temperature record 36.13 mean of scores, and there is a significant association between the study and control group post applying relaxation exercise (t=-5.470,p= 0.000). It is interesting to note the pulse rate for the study group was 72.90 mean of the score and the pulse rate for the control group was 70.40 mean of the score, and there is a significant association between study and control group (t=-2.232, p = 0.001. Finally, the respiratory rate was 14.70 mean of the score for the study group, and
15.83 mean of scores for the control group, and there is a significant association between the study and control group \((t=-3.554, p=0.001)\) after relaxation exercise applied to see table (2).

The comparison significance of vital signs means of score between pre-application and post-application period for the study group. Current findings show the SBP reading has a significant association between pre/post-test for the study group were \((t=4.054, p=0.000)\). Regarding DBP, the findings show a significant association between before applied period and after an applied period of relaxation exercise were \((t=2.948, p=0.000)\). Also, body temperature had a significant association between both pre/post applied relaxation exercises were \((t=11.366, p=0.000)\). According to pulse rate, there is a significance relationship pre/post applied relaxation exercise \((t=15.031, p=0.000)\). Regarding the respiratory rate, there is a significant association between pre/post applied relaxation exercise was \((t=16.858, p=0.000)\) see table (3).

### Table (1): Distribution of the (60) Diagnostic Clients According to the Demographical Characteristics and Divided to Study and Control.

<table>
<thead>
<tr>
<th>Basic Information</th>
<th>Groups</th>
<th>F total</th>
<th>% total</th>
<th>Study group N=30</th>
<th>Control group N=30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td>≤28</td>
<td>12</td>
<td>20</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>29-35</td>
<td>18</td>
<td>30</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>36-42</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>43-49</td>
<td>12</td>
<td>20</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>12</td>
<td>20</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Mean.sd=37.98 ±10.26</td>
<td></td>
<td>Mean.sd=37.90 ± 10.39</td>
<td>Mean.sd=38.06±10.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>18</td>
<td>30</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>42</td>
<td>70</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Mean.sd=37.98 ±10.26</td>
<td></td>
<td>Mean.sd=37.90 ± 10.39</td>
<td>Mean.sd=38.06±10.30</td>
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<td></td>
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<tr>
<td>Education Level</td>
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<td>13.3</td>
<td>4</td>
<td>13.3</td>
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<tr>
<td></td>
<td>secondary</td>
<td>8</td>
<td>13.3</td>
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<tr>
<td></td>
<td>Diploma</td>
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<td>Bachelor</td>
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<td>30</td>
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<td>26.7</td>
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<tr>
<td></td>
<td>Post-graduation</td>
<td>17</td>
<td>28.3</td>
<td>8</td>
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<td></td>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Mean.sd=37.98 ±10.26</td>
<td></td>
<td>Mean.sd=37.90 ± 10.39</td>
<td>Mean.sd=38.06±10.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
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<td>90</td>
<td>26</td>
<td>86.6</td>
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<tr>
<td></td>
<td>Single</td>
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<td>13.3</td>
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<tr>
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<td>Widow</td>
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<td>3.3</td>
<td>1</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>1</td>
<td>3.3</td>
<td>1</td>
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<tr>
<td></td>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Mean.sd=37.98 ±10.26</td>
<td></td>
<td>Mean.sd=37.90 ± 10.39</td>
<td>Mean.sd=38.06±10.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resident</td>
<td>Rural</td>
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<td>30</td>
<td>10</td>
<td>33.3</td>
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<tr>
<td></td>
<td>Urban</td>
<td>42</td>
<td>70</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>
Table (2): Comparison between Study and Control Groups’ Vital Signs at Post-applied of Relaxation Technique.

<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Study Group Mean ± Sd.</th>
<th>Control Group Mean ± Sd.</th>
<th>T-test</th>
<th>P. Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure</td>
<td>114.633 ±7.880</td>
<td>118.633 ±4.766</td>
<td>-2.379</td>
<td>0.020</td>
<td>S.</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>62.1333 ±1.851</td>
<td>65.000 ±3.912</td>
<td>-3.627</td>
<td>0.001</td>
<td>HS.</td>
</tr>
<tr>
<td>Temperature</td>
<td>37.066 ±0.583</td>
<td>36.133 ±0.730</td>
<td>-5.470</td>
<td>0.000</td>
<td>HS.</td>
</tr>
<tr>
<td>Pulse Rate</td>
<td>72.900 ± 4.389</td>
<td>70.400 ± 4.287</td>
<td>-2.232</td>
<td>0.030</td>
<td>S.</td>
</tr>
<tr>
<td>Respiration</td>
<td>14.700 ± 1.235</td>
<td>15.833 ± 1.234</td>
<td>-3.554</td>
<td>0.001</td>
<td>HS.</td>
</tr>
</tbody>
</table>

Sig. = Significance, S. = Significant, HS. = highly Significant Sd. = Standard deviation

Table (3): Comparison between Vital Signs Mean in (Pre and Post) Test to Study Group to Measure the Effectiveness of Applied the Relaxation Technique.

<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Pre-applied Mean ± Sd.</th>
<th>Post-applied Mean ± Sd.</th>
<th>T-test</th>
<th>P. Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure</td>
<td>118.6 ±4.825</td>
<td>114.633 ±7.880</td>
<td>4.054</td>
<td>0.000</td>
<td>HS.</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>65.1667 ±5.298</td>
<td>62.1333 ±1.851</td>
<td>2.948</td>
<td>0.000</td>
<td>HS.</td>
</tr>
<tr>
<td>Temperature</td>
<td>37.033 ±0.639</td>
<td>36.1333 ±0.730</td>
<td>11.366</td>
<td>0.000</td>
<td>HS.</td>
</tr>
<tr>
<td>Pulse Rate</td>
<td>72.966 ±4.468</td>
<td>70.400 ±4.287</td>
<td>15.031</td>
<td>0.000</td>
<td>HS.</td>
</tr>
<tr>
<td>Respiration</td>
<td>15.866 ±1.166</td>
<td>14.700 ±1.235</td>
<td>16.858</td>
<td>0.000</td>
<td>HS.</td>
</tr>
</tbody>
</table>

Sig. = Significance, S. = Significant, HS. = highly Significant Sd. = Standard deviation

Discussion

The current findings of 60 participants showed, according to SBP, there is a significant association 0.02 between the study group were the mean of score 114.6, and the standard deviation was 7.88. These results agree with the study done on preeclampsia primiparous women, the authors implement two types of exercise on two groups of preeclampsia woman and the found a significant association between the systolic and diastolic blood pressure reading in pre-intervention and post-intervention and there is no significant association in the control group [7]. These reflect the importance of relaxation exercise in reducing blood pressure (see table 2,3) This result of the study is similar with the article explained most people seeking medical attention for decreased blood pressure with muscle relaxation in various age group [8] and Maria et al. (2016) indicated that Simple yoga exercises (as relaxation exercises) may be useful as a supplementary hypertension therapy in addition to medical treatment [9]. Regarding temperature reading, the findings show the significance association
0.000, of body temperature, reading between pre-relaxation exercise intervention 37.066 ±0.583, and post-intervention 36.133 ±0.730, in comparison with pre-application of relaxation exercise for both study and control groups. These results come along with Ismansyah et al. they found the muscles relaxation have greatest effects on the body temperature in contrast with slow breathing intervention [10]. According to the pulse rate record, the results of the study show the significance association 0.030 between study group 72.900 ± 4.389 and the control group 70.400 ± 4.287, and the authors notice a slight alteration in pulse rate nearly two beats. On the other hand, there is no significant association between the study and control group before the application of relaxation exercise. These results are supported by Sahin & Basak were they found that can intervene progressive relaxation exercise as nursing interventions to increase patients’ satisfaction and positively affect vital signs in patients who undergo surgery with spinal anesthesia there is a significant association between study and control groups post- applied of progressive relaxation exercise. The importance of Sahin and Basak is evidence of the effects of relaxation exercise not only in reality but also in the virtual environment, so the current authors ensure the application of the relaxation exercise in a real environment. Finally, the respiratory rate also has light alteration after application of relaxation exercise where the p-value was 0.001 and the study group 14.700 ± 1.235 and the control group was 15.833 ± 1.234 and there is no significant association between study and control groups before relaxation exercise application [11]. These results come in the line with Hameed et al, and Chatterjee, & Mukherjee, they found the significant differences in the experimental group after they applied muscle relaxation exercise and music therapy [12&13]. The authors found the role of relaxation exercise to make a slight alteration in vital signs and especially in respiratory rate. Music as relaxation technique used to patients hospitalized in a hospice environment, they found this relaxation technique effect on vital parameters such as blood pressure, heart rate and oxygen saturation [14]. The result of this study similar the research done by Sarıtaş and Araç, (2016) explained the effectiveness of some relaxation therapy on vital signs (decrease in pulse values, systolic blood pressure value and diastolic blood pressure) and pointed the effectiveness of some relaxation therapy increase the oxygen saturation rate of patients in surgical intensive care unit [15].

Conclusions

The currents study concludes, some relaxation exercises such as (deep breathing, leg relaxation exercise, arm relaxation exercise, and face muscle relaxation exercise) after application for a study group, make a slight alteration in vital signs. And there is no significant association among the control group. The authors recommend the training of the office employees about some relaxation exercises.

Financial Disclosure: There is no financial disclosure.

Conflict of interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Adult of Nursing Department and all experiments were carried out in accordance with approved guidelines.

References


Effect of Diabetes and Hypertension on Right Carotid Artery Intima Media Thickness and Variable Spectral Waveform Indices And Parameters in Relation To Age for Iraqi Patients

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Abstract

Background: Arterial stiffness is related with atherosclerosis and cardiovascular disease events. Patients with atherosclerotic disease show to have larger diameters, reduced arterial compliance and lower flow velocities.

Aim of study: To compare between patients of two age groups with concomitant diseases diabetes and hypertension in regard to intima media thickness and blood flow characteristics in order to estimate the blood perfusion to the brain via the common and internal carotid arteries.

Subject and Methods: 40 patients with (diabetic and hypertension) diseases were enrolled, they were classified according to age. Color Doppler and B mode ultrasound was used to determine lumen Diameter (D), Intima – media thickness (IMT), end diastolic velocity (EDV), peak systolic velocity (PSV), Resistive Index (RI), Pulsatility Index (PI), and the flow rate indices were calibrated and calculated.

Results:- Results show that the change in the lumen diameter between the old patients group and the younger group were (2.02%) with insignificant (p value >0.05), and the difference in the in intima-media (IMT) thickness between the two age groups (35- 55) and (56-75) are (33.8%) with significant (p value <0.05). The change difference in peak systolic velocity was (-13.29%) with insignificant (p value >0.05), while the difference in the (EDV) between both age groups was (-38.02%), with significant p value <0.05. Pressure gradient and flow rate have given insignificant difference between both age groups.

Conclusion: The intima media is significantly thicker for the old age group than in the younger group. The increased thickness of intima media did not influence the lumen diameter significantly. A clear change in PSV, EDV, RI, and PI was observed which have influenced blood perfusion to the brain.

Keywords: Diabetic and hypertension, Blood flow velocity, Right common carotid artery.

Introduction

Several studies have shown that arterial stiffening occurs with age, and is associated with the increase of cardiovascular disease ¹,². Increased intima media thickness of the common carotid artery is a good marker of atherosclerotic and predictor incidence of stroke ³. Age effect on the particular smooth muscle (which found within and consist the majority of the wall of blood vessels, arteries have more bulk smooth muscle within their wall than vein, thus their greater wall thickness) can reduce elasticity and compliance, also increase in wall thickness, mainly affecting the media and intima, blood velocity also decreases, aged vessels show reduced collagen fiber and calcium are accumulated in the artery causing calcification and thus increase endothelial permeability.⁴

Diabetes and hypertension are a major risk factor for coronary heart disease and stroke and is the major
B-mode ultrasound high resolution which is non-invasive method was used to evaluated intima-media thickness of the common carotid artery can serve indicator for carotid atherosclerosis 6.

Internal carotid artery stenosis is estimated by parameters (end-diastolic velocity, peak systolic velocity, and resistive index) are used to measure the pulsatile blood flow that related to vascular resistance and vascular compliance caused by microvascular bed distal and reflects wall extensibility 7.

Pulsatility index can be calculated by (peak systolic velocity - end diastolic velocity) / mean flow velocity 8; increase pulsatility index may be indicate that severity of small vessel disease, contribute to vascular injury and progression of atherosclerosis in cerebral vasculature 9.

Doppler ultrasound was used to evaluate blood flow velocity in a cardiac cycle including end diastolic velocity (EDV), peak systolic velocity (PSV), pulsatility index (PI), and resistance index (RI) 10.

subject and methods

The study enrolled 40 patients with type 2 diabetes Mellitus and hypertension (21 females and 19 males, with a mean age of 56.17± 9.74 years). The consent of the patients were obtained for the purpose of conducting the study after being informed of the aim of the study and Medical Ethics Committee of hospital approved the study.

The present work was performed in National Diabetic center/ AL-Mustansyriah University, during the period May 2020 until July 2020.

Mindray (DC-60) Ultrasound and Doppler machine with a linear probe (7 L4A) at 6.6 – 13.5 MHz was used in this work.

All patients participating in the study suffering from hypertension and diabetes only.

The patient was lying in the supine position with aid of a pillow below the shoulder and neck to extend the neck and enable the examination of common and internal carotid arteries.

Intima-media thickness on the near and far wall (and mainly for far wall) of common carotid artery at both sides were selected and about 1 cm proximal to the bifurcation of the common carotid artery was measured. Lumen diameter was measured by placed a transducer at the lateral side of the neck correspond to the course of carotid artery.

Peak systolic velocity, End diastolic velocity, and Flow velocity were measured from both internal arteries, (1–2) cm distal to the bifurcation, the continuous tracing of the intimal-luminal interface of the near and far walls of the common carotid artery with at least three – five cardiac cycles to obtain optimum and clearest view for the wall and also for blood flow velocity and indices measurement.

Resistive index (RI) and Pulsatility index (PI) were measured and recorded.

Statistical analysis

Statistical data analyses performed using Microsoft Office Excel 2007.

Normally distributed continuous variables were presented as mean values ± standard deviation (SD). Differences between both age were analyzed with the unpaired t test. P value less than 0.05 was considered statistically significant.

Results

The study included 40 patients with hypertension and type 2 diabetes (21 females and 19 males), they have been divided into two age groups, the younger group age was 35-55 year old, mean age (47.7± 7.47 year) and the older group age was 56-75 year with mean age (62.43 ± 5.60 years). Table 1: shows the change difference between the two age groups 56-75 year old and young age group 35-55 year old for right common carotid and internal carotid arteries.

There was insignificant change in lumen diameter between the two age groups (2.02%), p value > 0.05. While the change in (IMT) between both age groups was (33.8%) with significant p value <0.05. The change in (PSV) was (-13.29%) was insignificant p value > 0.05. On the other hand the (EDV) change for younger and old
group (-38.02%) with significant value. The change in (RI) and (PI) between both age groups was (7.93%), (29.41%) respectively with significant p value < 0.05, and the change in pressure gradient and Flow rate for both age groups were insignificant p value > 0.05.

**Table 1:- The measured parameters for the right common & internal carotid arteries for two age groups using Doppler and B-mode**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(DM+HT) Patients (RCCA) Mean ±SD</th>
<th>Change % = ( \frac{\text{old-young}}{\text{young}} \times 100 )</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
<td>(35-55) years</td>
<td>(56-75) years</td>
<td></td>
</tr>
<tr>
<td>RCCA IMT (mm)</td>
<td>0.71±0.21</td>
<td>0.95 ± 0.29</td>
<td>33.8%</td>
</tr>
<tr>
<td>RCCA lumen D(mm)</td>
<td>5.44 ± 0.77</td>
<td>5.55 ± 0.944</td>
<td>2.02%</td>
</tr>
<tr>
<td><strong>Internal carotid artery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSV (cm/s)</td>
<td>40.85 ± 11.37</td>
<td>35.42 ± 11.47</td>
<td>-13.29%</td>
</tr>
<tr>
<td>EDV (cm/s)</td>
<td>16.7± 6.29</td>
<td>10.35±3.20</td>
<td>-38.02%</td>
</tr>
<tr>
<td>RI</td>
<td>0.63 ± 0.08</td>
<td>0.68 ± 0.061</td>
<td>7.93%</td>
</tr>
<tr>
<td>PI</td>
<td>1.02 ± 0.19</td>
<td>1.32 ± 0.293</td>
<td>29.41%</td>
</tr>
<tr>
<td>pressure gradient (mmHg)</td>
<td>0.72± 0.40</td>
<td>0.54±0.36</td>
<td>-25%</td>
</tr>
<tr>
<td>Flow rate (cm³/sec)</td>
<td>5.76±2.86</td>
<td>4.68±3.27</td>
<td>-18.75%</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study show that the change difference in intima media thickness for both age groups was (33.8%). It is expected that this increase in the thickness of intima media reduces the lumen diameter significantly which, in turn, reduce the blood flow but the overall change in the lumen diameter is very small between both groups, no more than (2.02%) in spite of change in the IMT thickness. This may be due to the arterial compliance decreases or increased arterial stiffness caused by aging, hypertension and diabetes is the result of changes in the artery wall, and also associated with increased collagen deposition due to increased wall thickness and elastin content of the intima declines, these changes in elastin and collagen content are important effects on the arteries stiffness; on the other hand age causes increase endothelial cell, and hypertrophy of vascular smooth muscle. The result show that the small change in the lumen diameter with a large change in the peak systolic velocity may indicate that the slower velocity did not relate to the change in the diameter of the lumen but it may be mainly linked with the increase in the flow resistance, this conclusion is reflected on the resistive index as it is higher in the elderly than younger group by 7.93% this index is an indicator of an increased resistance in the distal vessels. For this reason the reduction in peak systolic velocity in elderly is more than in the younger group by (-13.29%), table (1). In our study, the higher resistive index and lower end diastolic velocity for elderly group in patients with large artery atherosclerosis due to a greater resistance in intracranial
vessels that also associated with an increased risk of stroke and significantly increases the prediction of cardiovascular events.\textsuperscript{15,16}

We have observed a contralateral PSV and volume flow rate (VFR) in these cases gave a higher PSV and VFR for the young group than the old group, VFR is also higher for the younger group indicating less blood perfusion for the elderly.\textsuperscript{17} Result in the present study show that pulsatility index which is higher in elderly than younger group. This increase is related to increased distal vascular resistance, and a peaked waveform causes of blood flow to be more pulsatile (increased systolic flow and decrement diastolic flow), which induce fibrosis, calcification, hypertrophy of endothelium and smooth muscle cells in cerebral circulation, this led to marker of small vessel disease and microangiopathic changes in brain with subsequent of stroke events.\textsuperscript{18,19}

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science and all experiments were carried out in accordance with approved guidelines.

References


The Effect of Using Competitive and Traditional Methods in Learning Some Basic Gymnastics Skills

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Abstract

The research aims to identify the effect of using competitive and traditional methods on learning some basic skills in gymnastics. The research sample was selected from third stage students in the College of Physical Education and Sports Sciences at the University of Kufa. The research aims to identify the effect of using competitive and traditional methods on learning some basic skills in gymnastics. The researchers assumed that there were statistically significant differences between the pre and post-tests in favor of the post-test between the two methods separately, as well as the existence of statistically significant differences between the two methods in favor of the competitive method. In learning some basic skills in gymnastics, among the most important conclusions and recommendations made by the researchers are:

1- The group that learned the competitive method achieved an improvement in learning most skills in gymnastics than the group that learned the restrictive method.

2- Directing the attention of teachers and trainers in the sports field by using the competition method as a means of learning basic gymnastics skills.

Keywords: competitive style, learning basic gymnastics skills

Introduction

The effect of the teacher’s style on his students is the degree of their academic achievement, so teaching methods in the field of physical education should guide their teachers to the goals ¹, contents and methods that help in their success in teaching and educating students on scientific grounds because successful teaching includes a mature and useful scientific material for students. It is to be alive and fruitful so that the student interacts with it and benefits from it. It is not placed in the textbooks and methodology for the purpose of the student to read it or memorize it automatically and then return it in his theoretical and scientific tests. Rather, the scientific material studied by the student to think, contemplate, experiment, conclude and evaluate the results of his experience in it and in this way may become The scientific material is a culture in the student’s ² life and not just static information stuck in his memory, and the style of competition is one of the common and necessary methods of teaching, and the effectiveness of gymnastics is one of the activities that depend on achieving achievement on individual work and has received great attention to raising the level of artistic performance, and that this level is a result The process of motor learning is imperative ³. Much research has been conducted on the importance of competition methods in student education. Competition (self and between group members and groups) is an effective and powerful teaching method for influencing his working life.

Based on the above, Researchers decided to study one of the methods of competition, which is the method of competition and compare it with the traditional method of learning some basic skills in gymnastics, and the research problem was that the effectiveness of gymnastics is one of the individual sports activities included in the sports curriculum for the third stage in the Faculties of Physical Education and it includes a group One of the basic skills requires the student to learn them, and the researchers noted through their readings
and field observations that most of the methods used in teaching basic skills in gymnastics is the traditional method, which consists of the teacher’s self-views and ideas, directing the student’s work. From here the research problem emerged in the study of any of the two teaching styles, it is able to achieve the educational goals in a balanced manner and works to raise the level of students’ performance of basic skills in gymnastics in a better way. The objectives of the research were to identify the effect of using the competitive style and the traditional method in learning some basic skills in gymnastics and to identify the differences between the competitive and traditional styles in learning some basic gymnastics skills.

### Practical part

Researchers used the experimental method for its suitability in solving the research problem on students of the third stage in the College of Physical Education and Sports Sciences at the University of Kufa for the academic year 2019-2020 and the research sample consisted of students of the two divisions (B-C), which students (20 students) were randomly selected. As (10 students) from Division (B) were selected to represent the experimental group and (10 students) from Division (C) to represent the control group, and for the purpose of homogeneity, (2 students) were excluded from the research group due to absence and injury, and thus the research sample became (18 Student) and as indicated in Table (1).

#### Table (1). Shows the number of members of the two research groups and the teaching method used

<table>
<thead>
<tr>
<th>Class</th>
<th>groups</th>
<th>Teaching method</th>
<th>total number</th>
<th>sample members</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Experimental group</td>
<td>Competitive style</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
<td>control group</td>
<td>traditional method</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

For the purposes of homogeneity and parity between the two research groups in the age, weight and height variables, and in order for the researchers to return the difference to the experimental factor, the two research groups must be equivalent and homogeneous in the variables that are related to the research and which have an effect on the variable under study, so the process of parity and homogeneity between the research group was done to control the weight variables And age and height.

For the purpose of achieving parity between the two groups, research some basic gymnastics skills that have been identified, as shown in Table (3):
Table (2). shows the arithmetic mean, standard deviations and the value (calculated t) for the two research groups in some basic gymnastics skills that were covered by the research.

<table>
<thead>
<tr>
<th>Variables</th>
<th>unit of measurement</th>
<th>experimental group mean</th>
<th>standard deviation</th>
<th>control group mean</th>
<th>standard deviation</th>
<th>(t) calculate</th>
<th>Significant</th>
<th>Statistical Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>The forward roll opens</td>
<td>Degree</td>
<td>3.5</td>
<td>1.98</td>
<td>3.21</td>
<td>1.49</td>
<td>1.82</td>
<td>1.025</td>
<td>random</td>
</tr>
<tr>
<td>Rolling rear open</td>
<td>Degree</td>
<td>3.62</td>
<td>2.09</td>
<td>3.18</td>
<td>1.98</td>
<td>1.47</td>
<td>0.142</td>
<td>random</td>
</tr>
<tr>
<td>Handstand</td>
<td>Degree</td>
<td>3.63</td>
<td>1.26</td>
<td>3.58</td>
<td>1.35</td>
<td>1.20</td>
<td>1.210</td>
<td>random</td>
</tr>
</tbody>
</table>

Note that the value of (tabular t) is (1.74) with a degree of freedom (17) and a level of significance (0.05) greater than the value of (calculated t), and this indicates that there are no statistically significant differences between the two research groups, which indicates the parity of the two research groups,

**Tests used in the research**

Several basic motor skills have been selected in artistic gymnastics that are part of the gymnastics curriculum for third-stage students in the College of Physical Education and Sports Sciences. The most important of these skills are:

1- **Roll forward:**

It is the rotation of the body around its transverse axis so that its parts touch the ground from the shoulders to the pelvis and then the feet and lead in front of the body in a state of ball or curve and from stability or movement and that the centre of gravity of the body is close to the ground, which helps in the speed and ease of rolling.

2- **Roll back:**

It is the rotation of the body back around the axis of width and it is more difficult than rolling in front of the movement backwards and the presence of the head as an obstacle in this rolling and the movement leads backwards and the body is rounded or with the legs and they are extended and from different initial positions.

3- **Standing on the hands:**

Standing on the hands is one of the difficult movements because of the small base of support and the distance of the centre of gravity from the ground and the movement leads to either individual or doubles rise.

**Motor skills evaluation:**

Researchers relied on the opinions of experts specialized in artistic gymnastics on how to evaluate or give the final grade to students when they perform the motor skills assigned within the educational program, as it was agreed to take the degree of the subject’s professor (i.e. the final score that students obtain in the final exam when performing the motor skills).

On this basis, basic motor skills were evaluated in the students’ gymnastics hall.

**Exploratory Experience**

Researchers intended to conduct the exploratory experiment on a group of students from the same research community, but they did not enter the basic
experiment and the number reached (10) students. The experiment aimed to identify the obstacles and errors that could occur during the application of the program to avoid them, as well as the time taken for the tests.

**The main part of conducting research**

**The pretest**

Researchers intended to conduct the pre-test on the research sample in the gymnastics hall of the College of Physical Education and Sports Sciences at the University of Kufa, and Researchers intended to establish the conditions for testing from place and time and the assistant work team to achieve the same conditions or similar as possible when making the post-selection.

**Main experience (tutorial)**

The program included (12) educational units distributed into (3) basic skills in gymnastics, and each skill was allocated (4) lessons. Thus, the educational time for each skill reached (360) minutes, thus the total time for each group of the two research groups reached (1080 minutes), i.e. Equivalent to (18) hours, and the main experience (the educational program) lasted (45) days from (10/15 - 1/12/2019) and in the two teaching methods (competitive and traditional) knowing that the physical education study time takes (90) minutes in this program (The 90 minutes) broken down into:

- 25 minutes prep (warm-up)
- 45 minutes main part (tutorial application)
- 20 minutes closing part (calming down)

This division is applied to the two research groups and the implementation of the program was supervised by the assistant working group.

**Post-test**

After completing the application of the main experiment (the educational program), the post-test was conducted on the research sample on Thursday 3/12/2019 and under the same conditions or similar as possible for the test.

**Statistical means**

Researchers used statistical methods in the (SPSS) program

Results

Table (3). shows the arithmetic mean, standard deviations, and the value of (t) calculated in the pre and post-tests of the control group in some basic skills in gymnastics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>unit of measurement</th>
<th>Pre-test</th>
<th>Post test</th>
<th>(t) calculate</th>
<th>Significant</th>
<th>Statistical Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>STD.EV.</td>
<td>Mean</td>
<td>STD.EV.</td>
<td></td>
</tr>
<tr>
<td>The forward roll opens</td>
<td>Degree</td>
<td>3.21</td>
<td>1.49</td>
<td>5.27</td>
<td>1.42</td>
<td>3.92</td>
</tr>
<tr>
<td>Rolling rear open</td>
<td>Degree</td>
<td>3.18</td>
<td>1.98</td>
<td>5.96</td>
<td>1.46</td>
<td>3.55</td>
</tr>
<tr>
<td>Handstand</td>
<td>Degree</td>
<td>3.58</td>
<td>1.35</td>
<td>3.47</td>
<td>1.68</td>
<td>3.03</td>
</tr>
</tbody>
</table>

Note that the tabular value of (T) at the degree of freedom (8) with a level of significance (0.05) is evident from the results that appeared in Table (4) that the statistical differences were in favour of the dimensional test since all the calculated (t) values were greater than the values of (T) Tabular, which indicates the existence of a significant difference between the pre and post-test for the control group, which fulfils the first research hypothesis.
Table (4). shows the arithmetic mean, standard deviations, and the value of \( t \) calculated in the pre and post-tests of the experimental group in some basic gymnastics’ skills.

<table>
<thead>
<tr>
<th>Variables</th>
<th>unit of measurement</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>( t )</th>
<th>Significant</th>
<th>Statistical Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>STD.EV.</td>
<td>Mean</td>
<td>STD.EV.</td>
<td></td>
</tr>
<tr>
<td>The forward roll opens</td>
<td>Degree</td>
<td>3.5</td>
<td>1.98</td>
<td>7.97</td>
<td>1.54</td>
<td>6.62</td>
</tr>
<tr>
<td>Rolling rear open</td>
<td>Degree</td>
<td>3.62</td>
<td>2.09</td>
<td>6.21</td>
<td>1.28</td>
<td>5.21</td>
</tr>
<tr>
<td>Handstand</td>
<td>Degree</td>
<td>3.63</td>
<td>1.26</td>
<td>6.75</td>
<td>1.74</td>
<td>4.56</td>
</tr>
</tbody>
</table>

Note that the tabular value of \( t \) is at a degree of freedom (8) and with a level of significance (0.05) = (1.83). It is clear from the results that appeared in Table (5) that the statistical differences were in favour of the post-test since all the calculated \( t \) values were greater than Table (v) values, which indicates the presence of significant differences between the pre and post-tests of the experimental group, which fulfils the first research hypothesis.

Table (5). shows the arithmetic mean, standard deviations, and \( t \) value of the two research groups in the post-tests of some basic gymnastics’ skills.

<table>
<thead>
<tr>
<th>Variables</th>
<th>unit of measurement</th>
<th>experimental group mean</th>
<th>experimental group standard deviation</th>
<th>control group mean</th>
<th>control group standard deviation</th>
<th>( t )</th>
<th>Significant</th>
<th>Statistical Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The forward roll opens</td>
<td>Degree</td>
<td>5.27</td>
<td>1.42</td>
<td>7.97</td>
<td>1.54</td>
<td>2.95</td>
<td>0.000</td>
<td>Sig.</td>
</tr>
<tr>
<td>Rolling rear open</td>
<td>Degree</td>
<td>5.96</td>
<td>1.46</td>
<td>6.21</td>
<td>1.28</td>
<td>4.78</td>
<td>0.000</td>
<td>Sig.</td>
</tr>
<tr>
<td>Handstand</td>
<td>Degree</td>
<td>3.47</td>
<td>1.68</td>
<td>6.75</td>
<td>1.74</td>
<td>3.24</td>
<td>0.000</td>
<td>Sig.</td>
</tr>
</tbody>
</table>

Note that the tabular value of \( t \) is at the degree of freedom (17) and the level of significance (0.05) = (2.11). It is clear from the results that appeared in Table (6) that the calculated value of \( t \) was greater than the tabular value of \( t \), which indicates the existence of significant differences between the two groups in favour of the experimental group that used the competitive method, which fulfils the second research hypothesis.

Results

It is evident from the two tables (5 and 4) that there are significant differences between the pre and post-tests and in favour of the post-test, and the reasons for the differences between the two groups are attributed to the effect of the educational program. A proper understanding of the factors and principles that are relevant to the topic to prove their impact and value in certain educational situations. The results showed that there are significant differences in the level of students ’performance in all the basic skills chosen and in favour of the experimental group, which was trained and learned according to the competitive method among the group members. The
researchers attribute that to teaching The basic skill under the excitement and motives commensurate with the situations required by the basic skills during their practice in real competition and contribute to raising the level of skill performance which is included in the educational goal, and this is confirmed by (Lotfi Abdel Fattah) (The principle of kinetic learning calls that the practice and training must take place as much as possible. The way you will practice the skill (helping learners acquire skills more quickly). And that an atmosphere that is absolutely devoid of competition is unable to consult learners to learn and achieve, and that the atmosphere in which learners compete in friendship and sportsmanship is the best educational procedure, and (Barakat) confirms who mentions (that competition with a colleague in learning affects because it is a motivating factor and forces the learner to fully use his personality Notifying him of the results of his work, comparing him with his colleagues, and his notice of the extent of his progress or delay is one of the strongest motivations for learning and that the learner’s negligence and not notifying him of his position will lead the learner to boredom and slow learning).

**Conclusion**

There are significant differences between the pre and post-tests for the two teaching methods and in favor of the post-tests. The group that learned the competitive style achieved an improvement in learning most of the skills from the group that learned the traditional method. Every skill has a peculiarity in learning by imposing the kinetic style of the skill to be learned.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Ministry of education and all experiments were carried out in accordance with approved guidelines.

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Evaluation of Antioxidants Capacity of Non-Enzymatic Antioxidants and Its Effect in Glucose Level in Diabetic Patients

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Assistant Prof., College of Veterinary Medicine / University of Kerbala

Abstract

Background: Oxidative stress might participate in the pathophysiology of diabetes type 2. Systems of non-enzymatic antioxidants are made up of scavenging molecules that are formed endogenously.

The Study Objective: This study’s aims were evaluation of the non-enzymatic antioxidants and their effect in levels of glucose among diabetic patients.

Methodology: A total of eighty patients with DM type 2 and forty healthy persons (control) were enrolled within this study. Serum and plasma were obtained from collected blood samples. The Ferric Reducing Ability of Plasma (FRAP) method was used for detection of the total antioxidant capacity (TAC). Ellman’s method was used to determine the level of reduced glutathione. The serum CAT activity was detected by the technique explained by Sinha.

Results: The findings revealed that, among diabetic groups, there was a significant elevation in glycosylated hemoglobin and glucose levels; HbA1c and FBG levels were significantly higher; MDA, NO, LPI, TAC and GSH showed significant greater values. The activity of SOD showed significantly greater mean value among the control group.

Conclusion: The results suggest that antioxidants defense might be decreased in T2DM, as TAC levels were decreased. The increased levels of MDA, NO, LPI, TAC and GSH were associated with oxidative stress. Assessment of GSH could help in recognition of the extent of oxidative stress in diabetes as well as prevention and control of diabetic complications.

Keywords: Antioxidant, total antioxidant capacity, non-enzymatic antioxidants, diabetes

Introduction

Hyperglycemia that results from insulin dysfunction leads to interruption in the glucose homeostasis, one of these disorders is type 2 diabetes mellitus (T2DM). Diabetes is a chronic disorder that is, today, considered a common disease globally, and it is also widely spread among Arab countries 1. Recently, there are evidences suggesting that oxidative stress might participate in the pathophysiology of DM type 2 via rising the insulin resistance or making impairments in the secretion of insulin hormone 2. Regarding the fact that DM type 2 has an association with cardiovascular complications, oxidative stress was found to likely have a role in the diabetes pathogenesis as well as cardiovascular disease 3. Systems of non-enzymatic antioxidants are made up of scavenging molecules that are formed endogenously like glutathione, vitamin E, vitamin C, selenium, carotenoids, etc. 4. The imbalance amongst radical-generating and radical-scavenging systems is what causes oxidative stress which means more production of free radical or decrease in the antioxidant defense activity or both 5. It was reported that diabetes mellitus disease is along with an increase in the free radicals’ formation in addition to a decrease in the antioxidant defense capacity, resulting in an oxidative damage for cell elements 6. On the other hand, antioxidants are the line of defense that protects the body cells from the free radicals’ attack. The function...
of antioxidants is adding a large number of electrons into the blood vessels to be given to the monovalent free oxygen radicals that search for the lost electron, provided by antioxidants, so they become divalent, and hence, they settle and make no damage to the body cells. In diabetes, it was found that the levels of certain antioxidants like vitamin E as well as vitamin C were lowered. Additionally, among diabetic patients, the actions of antioxidant enzymes catalase, superoxide dismutase, as well as glutathione peroxidase was found to be diminished. Previously, numerous researches have been performed for studying the biomarkers of oxidative stress. In the early 1990s, a new total antioxidant capacity assay has been developed by Miller et al., and it was termed “total antioxidant capacity” (TAC).

**Methodology**

A total of 80 patients with DM type 2 and 40 healthy persons (control) were enrolled within the present study. Basic data such as age, gender, habits, lifestyle and previous medications were collected.

**Samples collection:**

Venous blood samples were collected from every fasted participant, within 2 various tubes, a plain tube contains sodium fluoride and another one EDTA coated. Samples that were collected in the plain tubes were centrifuged for obtaining the serum samples which were used for determination of antioxidant markers. Samples which were collected in EDTA coated tubes were utilized for determination of the glycated hemoglobin (HbA1c ≥ 6.2 0%) as well as levels of glucose in plasma.

**Measurement of the antioxidant markers:**

The Ferric Reducing Ability of Plasma (FRAP) method was used for determination of the total antioxidant capacity (TAC), where a ferric tripyridyltriazine complex of no color is reduced into a ferrous complex of blue color by the antioxidants present in serum. The findings were described in μmol per mg of protein. The Ellman’s method used to determine the level of reduced glutathione, via developing of a yellowish color which was read in the spectrophotometer at 412 nm. The results were expressed as μmol/mg protein. The serum CAT activity was detected by the technique explained by Sinha. The findings were described as U/mg of protein.

**Statistical Analysis**

Statistical analysis was done using SPSS (Statistical Packages for Social Sciences- version18). Data were analyzed via using One Way Analysis of Variance (ANOVA) for calculation of the p-value for healthy and other patients’ groups. Software Graph Pad Instat 3 was applied for testing the relation amongst the groups of the study. The results were presented as mean ± standard error. Statistical significant difference was considered at the level of (p ≤ 0.05).

**Results**

The basic data of the study participants were shown in Table 1. Among the study groups, males appeared to be more than females, where the sex ratio was [1.09]. The patients had an age ranged from thirty to ninety years with mean of [54 ± 3] years. The age represented was mostly from forty to sixty among healthy people as well as the diabetic patients, resembling (75%) and (60%) respectively.

**Table 1: Basic data of the sample population:**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Diabetic patients N= 80(%)</th>
<th>Control N= 40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>47.5</td>
<td>40</td>
</tr>
<tr>
<td>Male</td>
<td>52.5</td>
<td>60</td>
</tr>
<tr>
<td>30&lt; age&lt;40</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>40&lt; age&lt;50</td>
<td>27.5</td>
<td>40</td>
</tr>
<tr>
<td>50&lt; age&lt;60</td>
<td>32.5</td>
<td>35</td>
</tr>
<tr>
<td>60&lt; age&lt;70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>&gt;70</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>


**Variation of clinical parameters:**

Fasting blood glucose (FBG) and Glycated hemoglobin levels were detected for evaluation of the glycemic state among diabetic cases, they were shown in Table 2.

and FBG mean ± SD=(2.12 ± 0.14, and 1.14 ± 0.04, respectively). The body mass index (BMI) values had no significant change among patients and control groups.

| Table 2: Clinical parameters variation between control and diabetic groups: |
|-----------------------------|-----------------------------|-----------------------------|
|                            | Diabetic patients (mean ± SD) N = 80 | Control (mean ± SD) N = 40 | P value   |
| BMI (kg m²)                | 27.21 ± 5.27                 | 29.03 ± 5.32                | P > 0.05  |
| HbA1C (%)                  | 8.21 ± 0.45                  | 4.57 ± 0.11                 | P < 0.05  |
| FBG (g/l)                  | 2.12 ± 0.14                  |                             |           |

**Variation of markers of oxidative stress:**

The current study also reveals that there were modifications within both oxidant and antioxidant conditions of patients compared to controls.

The activity of superoxide dismutase (SOD) showed significantly greater mean value among the control group (0.42 ± 0.04) compared to the diabetic patients (0.37 ± 0.03) (P < 0.05). Malondialdehyde (MDA) is an aldehyde of low molecular weight which could be resulted from the attack of free radical on polyunsaturated fatty acids of biological membranes and its detection is useful in examining lipoperoxidation. The levels of serum MDA, nitrite oxide (NO) and lipid peroxidation index (LPI) showed greater values among diabetic patients (0.28 ± 0.02, 216.06 ± 8.95, and 0.182, respectively) than control group (0.29 ± 0.03, 191.40 ± 11.54, and 0.181, respectively) which indicate the oxidative damage of lipids mediated by free radicals.

The values of TAC showed a significant increase (P < 0.05) among diabetic patients (160.45 ± 7.64) than control group (210.47). In addition, the reduced glutathione (GSH) had significantly higher values (P < 0.05) in diabetic patients (9.79 ± 0.59) than control group (6.76 ± 0.58), Figure 1.
Figure 1: Levels of oxidant and antioxidant markers.

(MDA malondialdehyde, NO nitrite oxide, TAC total antioxidant capacity, SOD superoxide dismutase, GSH reduced glutathione, LPI lipid peroxidation index. * significant at P < 0.05)

Also, a significant negative relation between FBG and glutathione were detected in diabetic patients (P < 0.05), while HbA1 showed a significant positive correlation with NO among diabetic patients (P < 0.05) indicating that oxidative stress might influence HbA1c, Table 3.

Table 3: Correlation between clinical parameters and antioxidant in control and patients’ group:

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (mean ± SD) N = 80</th>
<th>Control (mean ± SD) N = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>P value</td>
</tr>
<tr>
<td>HbA1 and NO</td>
<td>0.74</td>
<td>0.003</td>
</tr>
<tr>
<td>FBG and CAT</td>
<td>−0.152</td>
<td>0.340</td>
</tr>
<tr>
<td>FBG and glutathione</td>
<td>−0.541</td>
<td>0.001</td>
</tr>
<tr>
<td>TAC and glutathione</td>
<td>0.123</td>
<td>0.440</td>
</tr>
</tbody>
</table>

Discussion

Oxidative stress might participate in the pathophysiology of diabetes type 2 via rising the insulin resistance or making impairments in the secretion of insulin hormone [2]. In T2DM, the oxidative stress had an association with massive alterations on the systems of antioxidant enzyme (SOD, CAT, GPx, GSH) as well as total antioxidant capacity (TAC) that result in peroxidative damage for proteins, lipids, carbohydrates, and the nucleic acids, as well that could be used as biomarkers for diabetes [3].

The current study assessed the oxidant status, HbA1c as well as FBG in a group of patients with T2DM. The age group which was represented mostly was between
[forty to sixty] for healthy persons as well as the diabetic patients.

HbA1c and FBG levels of diabetic patients were significantly higher than healthy people. These results were in agreement with Abudawood et al. 14 who has performed a study on the association between oxidative stress, DNA damage and cancer risk in T2DM in Riyadh, KSA, and higher levels were found of HbA1c and FBG in diabetic patients compared to controls. HbA1c and FBG are the indicators of type2 diabetes mellitus where the elevated glucose levels interfere with the metabolism of the free radicals and this is associated with increasing in the lipid and protein oxidation leading to more tissue damage 15,16.

The current study showed that diabetes mellitus disease, hypertension as well as smoking increases the oxidative stress which is represented by the significantly lower mean value of the activity of SOD among the diabetic patients, and significantly higher levels of MDA, and NO that were shown among diabetic patients, (P < 0.05). These findings were in agreement with the findings of Pieme et al. 17 study. MDA is the most essential marker of oxidative stress 18.

Raddam et al. 18 also revealed that the levels of MDA had significant high values and lower SOD levels in all diabetic patients. Also, Evereklioglu 19 found that patients with diabetes with macular degeneration showed a lesser SOD activity and greater of MDA and NO levels compared to healthy persons.

Additionally, this study revealed a significant reduction in the total antioxidant capacity (TAC) with a higher lipid peroxidation index (LPI) (but it was non-significant) in diabetic group.

A study by Sohrab et al. [20] on men with T2DM showed similar findings; where it was found that higher levels of markers of oxidative stress were observed with lower TAC. McCracken et al. 21 also found that the TAC levels were reduced among diabetic patients without glycemic control, and this was partially improved with suitable glycemic control. Similarly, Čolak et al. 22 revealed that the total antioxidant capacity were significantly lower among diabetic patients. It was mentioned that the decrease in the levels of the total antioxidant capacity is correlated to a higher occurrence of diabetes disease as well as its complications 23.

Reduced GSH, is a non-enzymatic antioxidant that has a significant role via making protection of the cells from the oxidative damage and, consequently, maintain the levels of the active state of vitamins E and C within cells throughout making neutralization to the free radicals 4. There is a possibility that the elevation in the GSH levels among diabetic patients is correlated to increasing the glutathione peroxidase activity by neutralizing free radicals that were created in the disease complications. It was found that the reduced GSH, an extracellular non-enzymatic antioxidant, makes inhibitions to the oxidative activity via several ways 27. The increased levels of NO in addition to the positive correlation between NO and HbA1 observed in diabetic patients could be described by the theory of the effect of hyperglycemia on the endothelium of blood vessels and the related vascular tone through influencing levels of NO 28. This study revealed that the oxidative stress markers were increased in diabetic patients with decreased SOD. In an experimental study, the kidney and heart SOD levels as well as liver GPx were increased 29.

**Conclusion**

This study aimed to evaluate the non-enzymatic antioxidants and its effect on levels of glucose among diabetic patients. From the findings of this study, it is suggested that there is some of evidence that antioxidants defense might be decreased in T2DM, as serum TAC was decreased. In addition, the activities of SOD were found to be reduced in diabetic patients while MDA, NO, LPI, TAC and GSH showed higher values among diabetics. The increased NO levels identifies the effect of hyperglycemia on the vascular endothelium and increasing the risk of cardiovascular complications of T2DM due to decreasing the vascular antioxidant defense. In addition, the increased levels of GSH in T2DM support that fact that oxidative stress might participate in the pathophysiology of diabetes type 2. Therefore, it is recommended that assessment of GSH could help in recognition of the extent of oxidative stress in diabetes as well as prevention and control of diabetic complications.

**Financial Disclosure:** There is no financial disclosure.
**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Veterinary Medicine and all experiments were carried out in accordance with approved guidelines.

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Measurement of GSH, SOD and MDA some Antioxidant level after Excises Athletes

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2 Prof Dr. Iraq\ Almustaqbal University College - Department of Physical Education and sport Science,
3 Prof. Dr. , Iraq, AL-Qasim Green University. College of Physical Education and sport Science

Abstract

Antioxidants are a line of defense that protects the cells of the body. Attacking the stray oxygen molecules. The function of these antioxidant is to add a group of electrons to the blood vessels to give them to electrolytes or monovalent free radicals so that they stabilize and calm down and do not destroy cells. Thus, antioxidants work to achieve a balance between free radicals and antioxidants In the cell and keeps the cell from being damaged. Increased electrolytes or free radicals work To weaken the ability of antioxidants’ and enzymes secreted by cells despite the fact that the cell has a self-protection and a defensive line to secrete self-antioxidants, and here the importance of antioxidants arises. These metabolic changes increase the oxidative effort and affect the ability to achieve and the efficiency of athletes, as well as the body contains a lot of antioxidant systems Oxidative stress that includes enzymes such as GSH) Glutathione Dos) Super oxide D ismutses. Exercise can produce an imbalance between ROS and antioxidants, which is referred to as oxidative stress. Dietary antioxidant supplements are marketed to and used by athletes as a means to counteract the oxidative stress of exercise. Whether strenuous exercise does, in fact, increase the need for additional antioxidants in the diet is not clear. This research showed the role of free radicals in causing oxidative stress during exercise. The results showed significant increased in malondialdehyde (MDA) level (as an index of lipid peroxidation) glutathione (GSH) superoxide dismuatse activity (SOD) in subjects after efforts of exercise in comparison with the control group.

Key Word : SOD, antioxidant , MAD, Athletes, Sport

Introduction

The cells in our body continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. Free radicals are molecules or part of molecules which have one or more unpaired electrons in external electronic shell. Main characteristics of these molecules are very short life span and extremely high reactivity. Injurious effects of free radicals are induced by necessity to establish electronic stability and therefore they react with next stable molecule, taking its electron and creating new free radical. The at way this molecules also becomes unstable and further interferes with other molecules from its surrounding which leads to impairments of cellular components. Free radicals are created during the process of oxidative phosphorylation in mitochondria 14. Oxidative stress occurs as a result of ROS activity and reduced protective mechanisms that lead to impairments in cells and tissues functions. It causes secondary damage through late cell death and inflammation 15. Various studies have shown that oxidative stress represents pathogenetic foundation of many diseases 16. ROS are normally neutralized by complex system of antioxidant defence 17. The system of antioxidant defence can be divided into two groups: enzymes including superoxid dismutase (SOD), catalase (CAT), glutathione preoxidase (GPX); and non-enzymes including vitamins C and E, retinol, bilirubin, uric acid, redox glutathione, thiols, coenzyme Q₁₀, stress proteins, albumins, as well as transport proteins and storage proteins for Fe²⁺ i Cu²⁺ which disable potentially harmful metal ions and their involvement in
production of free radicals. Nevertheless, low levels of ROS appear to be necessary for important physiological functions such as cell signaling, immune response, and apoptosis. Many studies have shown that exercise induces oxidative stress and causes adaptations in antioxidant defenses. Training can have positive or negative effects on oxidative stress depending on training load, training specificity and the basal level of training. Data suggest that regular long term training can induce antioxidant response to the oxidative stress. The results of a study which investigated the relationship between oxidative stress and exercise overtraining/overreaching support the possibility that the beneficial effect of physical exercise on oxidative stress might be associated with increased antioxidant defences. It is also well known that active and non-active skeletal muscles produce reactive oxygen and nitrogen species although it is not quite clear where oxidants originate during physical activity. The degree of oxidative damage, as well as the time course for elevation in oxidative stress markers has varied across studies, and appears to be dependent, among all, on the type, intensity, volume and duration of exercise. This leads to differences in oxidative status between athletes in different sport disciplines, but the results of the previous studies are inconsistent.

**Subjects and Exercise Programmed:**

12 male student volunteers as Control from education college– from Almustaqbal university college, were recruited to participate in the study. And 45 male volunteers athletes from different gym sport in Babylon province, ages between (20-40) years. All subjects were The questionnaire taken from the case and control sheets involved: types of food, occupation, age, type exercise, gender, Supplements, smoking habit, alcohol intake type Sport. All tests took place in human performance laboratory of Almustaqbal university college. The temperature ranged from 14°C to 20°C. Athletes performed all testing in the same equipment conditions and drank the same energy beverage.

**Blood Sampling Procedures**

About (5 ml.) Blood samples were collected by puncture from an antecubital vein in resting after Exercise conditions The blood samples were centrifuged (at 3000 rpm for 300 sec), and serum was divided into aliquots and frozen in dry ice prior to storage at –20°C until assayed for glutathione, MDA, and superoxide dismutase.

**Antioxidant Analysis**

**Determination of Reduced Glutathione(GSH) in Blood Serum**

GSH level was determined in blood serum using a modified method dependant by Sedlack and Lindsay, (1968). The method depends on Ellman’s reagent which contains [5,5-dithiobis(2-nitrobenzoic acid)] or known as DTNB which reacts with the thiol group of reduced glutathione to form a coloured solution absorbed at (412) nm.

**Determination of Lipid Peroxidation in Blood Serum (Malondialdehyde, MDA)**

MDA level was determined in blood serum through the measurement of MDA concentration. The method depends on the reaction between MDA and thiobarbituric acid or (Beruge and Aust, 1978) to form a coloured solution absorbed at (532) nm.

**Determination of Superoxide Dismutase (SOD) Activity in Blood Serum**

(SOD) activity level in blood serum was determined using photochemical method. The method included using sodium cyanide as peroxidase inhibitor. This methods depends on an indirect approach to determine the SOD activity through the change in formazene absorbance formed from the reduction of O2 • (which is produced by radiating the sample of serum with light) for nitroblue tetrazolum (NBT) dye (Brown and Goldstein, 1983). Decreased difference in formazene absorbance means increased SOD activity.

**Data Analysis**

All values are presented as means ± SD. Between-group, differences for selected variables were determined by SPSS Statistic Analysis.
Results and Discussion

Table 1 show the relationship in Parameters of Antioxidant level enzyme between the control and case according the Job.

<table>
<thead>
<tr>
<th>JOB</th>
<th>Parameters of Antioxidant level enzyme</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH μmol/L</td>
<td>SOD μmol/L</td>
</tr>
<tr>
<td>Student</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.5380</td>
<td>165.3750</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.88014</td>
<td>158.5280</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.31118</td>
<td>5.58366</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.9425</td>
<td>212.0833</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.48931</td>
<td>21.16368</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.14125</td>
<td>6.10943</td>
</tr>
<tr>
<td>Non Employ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.0472</td>
<td>186.3000</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.24248</td>
<td>171.4722</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.27783</td>
<td>38.34237</td>
</tr>
<tr>
<td>Employ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.9095</td>
<td>180.0000</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.25936</td>
<td>84.85281</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.89050</td>
<td>60.00000</td>
</tr>
</tbody>
</table>

Table 2 show the relationship in Parameters of Antioxidant level enzyme GSH SOD and MAD between the control and case according the Type Sport.

<table>
<thead>
<tr>
<th>TYPE</th>
<th>Parameters of Antioxidant level enzyme</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH μmol/L</td>
<td>SOD μmol/L</td>
</tr>
<tr>
<td>Athletes</td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>4.3744</td>
<td>228.1000</td>
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<tr>
<td>Std. Deviation</td>
<td>.62194</td>
<td>143.49019</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.19668</td>
<td>45.37558</td>
</tr>
<tr>
<td>Building body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.1661</td>
<td>156.4000</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.35567</td>
<td>169.76591</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.30314</td>
<td>37.96081</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.9425</td>
<td>212.0833</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.48931</td>
<td>21.16368</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.14125</td>
<td>6.10943</td>
</tr>
</tbody>
</table>
Table 3 show the relationship in Parameters of Antioxidant level enzyme GSH SOD and MAD between the control and case according the taken Protein.

<table>
<thead>
<tr>
<th>PROTIEN</th>
<th>Parameters of Antioxidant level enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH μmol/L</td>
</tr>
<tr>
<td>No protein</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.8762</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.19026</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.28055</td>
</tr>
<tr>
<td>Have protein</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.7745</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.89422</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.25814</td>
</tr>
</tbody>
</table>

Table 4 show the relationship in Parameters of Antioxidant level enzyme GSH SOD and MAD between the control and case according the Smoker.

<table>
<thead>
<tr>
<th>SMOKER</th>
<th>Parameters of Antioxidant level enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH μmol/L</td>
</tr>
<tr>
<td>Non Smoker</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.2071</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.24584</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.25431</td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.3492</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.76080</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.31060</td>
</tr>
<tr>
<td>Control non Smoker</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.9425</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.48931</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.14125</td>
</tr>
</tbody>
</table>
Table 5 show the relationship in Parameters of Antioxidant level enzyme GSH, SOD and MAD between the control and case according the Nergla Smoker.

<table>
<thead>
<tr>
<th>Nergela SMOK</th>
<th>Parameters of Antioxidant level enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH μmol/L</td>
</tr>
<tr>
<td><strong>No smoker</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.3333</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.65906</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.14382</td>
</tr>
<tr>
<td><strong>smoker</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.7716</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.95318</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.61765</td>
</tr>
<tr>
<td><strong>Control no smoker</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.9657</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.46054</td>
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<tr>
<td>Std. Error of Mean</td>
<td>.17407</td>
</tr>
<tr>
<td><strong>Control smoker</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.9750</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>.65000</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>.32500</td>
</tr>
</tbody>
</table>

Table 6 show the Correlation in Parameters of Antioxidant level enzyme GSH, SOD and MAD in the case according the Type Sport.

<table>
<thead>
<tr>
<th>Correlations type of sport (football, body sport, control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of Squares</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>SOD μmol/L</strong></td>
</tr>
<tr>
<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
<tr>
<td><strong>MAD μmol/L</strong></td>
</tr>
<tr>
<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
<tr>
<td><strong>GSH μmol/L</strong></td>
</tr>
<tr>
<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
</tbody>
</table>
The increase in energy consumed during exercise increases the oxygen demands of the active tissues, increasing up to 20 times in comparison with basal state. The oxygen flow in the peripheral skeletal muscle tissue can increase up to 200 times, increasing 30 times the blood flow, and the oxygen difference in the arteriovenous blow increases 3 times. As a result, the oxidative metabolism is increased, maximizing the energy produced by unit of substrate and avoiding lactate accumulation. Dillard et al. (1978) first described that extenuant exercise induced lipid damage in tissues. After that, many other investigations focused on the effects of exercise and training in oxygen toxicity and the body defense response. It is accepted that oxygen toxicity can be implicated in some pathologic situations. The understanding of the mechanisms associated with physiological responses that explain how exercise increases the oxygen toxicity and the design of appropriate measures to minimize toxicity are indispensable to: 1. Increase exercise efficacy as a preventive and therapeutic instrument in clinical practice. 2. Control the damaged tissue induced by exercise. Oxidative stress induced by extenuant exercise is a situation by which cells are exposed to a prooxidant environment and defense mechanisms are not enough, affecting the redox state of the cells. Due to this, nutritional supplements of antioxidants such as vitamin C, vitamin E, carotenids, and polyphenols in the diet are important.

Further research is required to support these hypotheses. Where the hypotheses said the Malondialdehyde (MDA) is one of the most frequently used indicators or biomarkers of lipid peroxidation. It was significantly increased as a result of exercise in the present study. Two theories support the concept that resistance exercise could lead to an increase in the production of oxygen free radicals in active muscle sites. A widely held hypothesis involves the ischemia-reperfusion injury. Intense muscle contractions can result in a temporary decrease in blood flow and oxygen availability and subsequent ischemia. The following reperfusion period (muscle relaxation) produces an abundant reintroduction of O2 and results in the formation of the O2- radical. Mechanical stress is another hypothesis used to explain an increase in free radicals. In particular, eccentric exercise causes high levels of force that has been shown to initiate muscle tissue damage. This initiates the inflammation process that eventually produces oxygen free radicals and lipid peroxidation. The significant increase in MDA agrees with other investigators. They reported that moderate intensive treadmill running exercise was sufficient to result in muscle damage and increases in the susceptibility of erythrocytes to in vitro peroxidation represented by MDA. Similar result was reported by others where they suggested an increase in plasma MDA immediately after exercise. It was also recorded a significant increase in MDA at 6 hours post exercise. Glutathione (GSH) oxidation in different tissues is a valid parameter to appreciate oxidative stress. In this situation, intracellular GSH rapidly oxidizes to GSSG. Intracellular GSSG can be reduced to GSH in the presence of a reductase glutathione and NADPH as cofactor. When the oxidative stress is high, the relation between GSSG/GSH can be higher than the reduction ability of the cells. In this situation, the heart and skeletal muscle cells pour GSSG out of the cells. In extenuant exercise, an increase of GSSG and a decrease of total glutation (GSSG + GSH) in the skeletal muscle tissues such as the liver and heart has been observed. This increasing production of GSSG exceeds the reductase glutathione’s ability to reduce disulfide group, thus explaining that the GSSG spill from the tissue to the plasma. The increasing oxidized glutathione plasma concentration as a result of the exercise has been demonstrated in many studies.

The glutathione synthesis ability in the liver is high and exercise induces a decrease of glutathione, promoting a protective response of the liver. Up to now, the work has been focused in the damaging effect of exhaustive exercise. However, moderate exercise results in a healthy and beneficial practice that prevents diseases, due to its ability to prevent oxidative stress. Oxidative stress induced by exercise depends on the type, intensity, and the length of the exercise. However, interindividual variability is attributed to the level of training, sex, nutrition, and genetic factors.

In biological tissues, the superoxide anion can be converted into the nonradical species hydrogen peroxide and singlet oxygen by the aid of SOD enzyme. Thus, SOD provide the primary defense against ROS generated during exercise. The activity of this enzyme is known to increase in response to exercise in both animal and human studies. Like to the present study in which SOD activity was increase significantly (p<0.001) in training athletes.
Therefore, athletic performance increases the production of free radicals that lead to cell damage, and then it has been observed that physical performance leads to an increase in the blood level as well as an increase in the expiratory air content of the lungs, both of which are indirect signs of metabolic oxidation, even if they differ in different people. Take an anti-oxidant Oxidation during food or through preparations before training reduces muscle fibers resulting from athletic training. A decrease in the level of fat in the blood was observed as a result of an increase Training time due to increased adaptation, Also, an increase in the blood content of the reduced GSH image was observed. In another hand, it was found that running training for athletes improves the anti-oxidant amount of blood compared to non-athletes in terms of the red blood cell content of Vitamin E for Glutatione and the activity of the catalase enzyme. So this research show the Regular exercise increases the efficiency of the antioxidant defense system Reduced amount of oxidative stress in the absence of regular exercise.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Almustaqbal University College and all experiments were carried out in accordance with approved guidelines.

References


Health Consequence of Gas Filling on the Workers’ Health of Two Cities in Iraq (Comparative Study)

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Abstract

A gas is a sample of matter that conforms to the shape of a container in which it is held and acquires a uniform density inside the container, even in the presence of gravity and regardless of the amount of substance in the container. If not confined to a container, gaseous matter, also known as vapor, will disperse into space. The term gas is also used in reference to the state, or condition, of matter having this property.

Aims: To identify the demographic data of the worker and to comparative beteen kirkuk gas filling company and Tikrit gas filling company among work hazards like health, physical, chemical, physiological, mechanical hazards.

Methodology: Quantitative design (comparative study) was conducted for worker in company gas filling in Tikrit (Salah aldin) and Kirkuk city the study was carried out from the 10th February 2020 to 13th August 2020. This study was conducted in Kirkuk city and Tikrit (Salah aldin). The present study was conducted in two company of gas filling: Company gas filling / branch Kirkuk and Company gas filling / branch salah Eldin. The data was prepared, organized and coded into the computer file; Statistical Package for Social Science (SPSS) version (24) was used for data analysis

Results the result of demographic data for both kirkuk and Tikrit city shows that the age group (20-29 years) represents a high percentage in the Kirkuk gas and Tikrit city and represent (40.0%) and (38.0%) respectively, with regard to the gender the results shows high percentage from gender were male in kirkuk and tikrit and represent (95.0%) and (98.0%) respectively, also the result shows the kirkuk workers were more less effected than tikrit workers also the results shows the chemical, mechanical and psychosocial hazard was effect in tikrit workers more than kirkuk workers.

Key word: Health consequence, Gas Filling, workers health

Introduction

A gas is characterized as a detail of issue comprising of particles that have neither a characterized volume nor characterized shape. It is one of the four essential conditions of issue, alongside solids, fluids, and plasma. Under customary conditions, the gas state is between the fluid and plasma states. A gas may comprise of molecules of one component (e.g., H₂, Ar) or of mixes (e.g., HCl, CO₂) or blends (e.g., air, characteristic gas) (1) Flammable gas is utilized in numerous homes for warming and cooking. Lamentably, petroleum gas holes may happen without the mortgage holder in any event, acknowledging there is a gas spill. A few people’s feeling of smell is extremely delicate to gaseous petrol, while others can’t smell it by any means. Seeing a portion of the notice indications of a petroleum gas spill in the home might just spare a daily existence (2) Cerebral pains and Dizziness, for some people, the primary indication of a gas break might be a migraine. While huge numbers of us experience migraines every day, unexpected or unexplainable cerebral pains ought to never be disregarded. On the off chance that the migraine doesn’t disappear after you go outside for
some time, or while you’re grinding away, consider the possibility that you may have a flammable gas spill. People may encounter episodes of dazedness alongside a cerebral pain. Ask other relatives or flat mates in the event that they’re encountering episodes of cerebral pains and dazedness also (3) Queasiness, Along with migraines and wooziness, people presented to a gaseous petrol hole may likewise encounter sickness. Such manifestations might be exacerbated if the house is quit for the day winter, or if the carport or storm cellar isn’t appropriately ventilated. Sporadic Breathing, A flammable gas hole may cause trouble or unpredictable taking in a few, particularly the youthful and older. note that a gaseous petrol break may suck oxygen from the air and produce substantial measures of carbon dioxide, which can prompt trouble breathing that may diminish the measure of oxygen in your lungs and blood and cause obviousness. Weariness, Some people presented to a petroleum gas spill additionally feel a mind-boggling feeling of exhaustion or dormancy. As a rule, this is brought about by absence of sufficient oxygen stream in the body. An individual ought to recuperate rapidly whenever moved to an alternate area. (4)

Methodology

Quantitative design (comparative study) was conducted for worker in company gas filling in Tikrit (Salah aldin) and Kirkuk city the study was carried out from the 10th February 2020 to 13th Augst 2020

This study was conducted in Kirkuk city and Tikrit (Salah aldin ). The present study was conducted in two company of gas filling: Company gas filling / branch Kirkuk and Company gas filling / branch salah Eldin.

The Sample of the study consist of from a non-probability (purposive) sample was selected. It consisted of (200) workers .(100) workers of sample was collected from mechanical Company gas of Kirkuk City and (100) manual company gas of mechanical Company gas of Salah Eldin City.

The study instrument (questionnaire) was consisted of three major parts to meet the purposes of study. The first part is related to workers demographical characteristics such as (age, gender, residence, marital status, department, educational attainment, monthly income, years of service, time of work per day, training, and number of training), the second part is related to Health Hazards these part include Physical health hazards: They consist of (9)items ,chemical health hazards, they consist of (8) items ,mechanical health hazards: they consist of (7) items ,Psychological health hazards: They consist of (5) items and biological health hazards: They consist of (5) items and the third part is related to precautionary measures They consist of (11) items . The overall items were ( 56) items, by using the scale (Agree=3), (Neutral =2), (Disagreement =1). Content validity was determined by presenting the questionnaire to a panel of (11) experts in different specializations, six in medical surgical nursing, three in community health nursing, and two in maternity and child health .The data collection was carried out for the period from the 10th February 2020 to 10th April 2020

The data was prepared, organized and coded into the computer file; Statistical Package for Social Science (SPSS) version (24) was used for data analysis

Result of the study :-

Table (1) : Distribution of the sample according to demographic characteristics (200) samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>frequency</td>
<td>percentage</td>
</tr>
<tr>
<td>20-29yrs</td>
<td>40</td>
<td>40.0</td>
</tr>
<tr>
<td>30-39yrs</td>
<td>26</td>
<td>29.0</td>
</tr>
<tr>
<td>40-49 yrs</td>
<td>19</td>
<td>19.0</td>
</tr>
</tbody>
</table>
Table (1) show results The demographic data for both Kirkuk and Tikrit city the result ashows that the age group (20-29 years) represents a high percentage in the Kirkuk gas and Tikrit city and represent (40.0%) and (38.0%) respectively , with regard to the gender the results shows high percentage from gender were male in kirkuk and tikrit and represent (95.0%) and (98.0%) respectively .also the result shows high percentage from workers was worked at direct department contact in kirkuk and tikrit and represent (78.0%) and (82.0%) respectively ,finally the time of work per day the results shows the worker were work from(6-8)hrs per day and represnt (78.0%) and (73.0%) in kirkuk and tikrit respectively .
Table (2) Comparative about awareness of gas station workers regarding physical hazard at at Kirkuk city and Tikrit city

<table>
<thead>
<tr>
<th>No</th>
<th>items</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Assess of awareness</td>
</tr>
<tr>
<td>1</td>
<td>The noise level in my work place is relatively high</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>2</td>
<td>Loss of hearing could result from exposure to load noise</td>
<td>2.7</td>
<td>HA</td>
</tr>
<tr>
<td>3</td>
<td>My job function has to do with working with object, tool, equipment, machine, chemical, that has high temperature</td>
<td>2.7</td>
<td>HA</td>
</tr>
<tr>
<td>4</td>
<td>Extreme heat could cause body cramp</td>
<td>2.5</td>
<td>HA</td>
</tr>
<tr>
<td>5</td>
<td>When you carry gas bottles you feel back pain</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>6</td>
<td>Vibration could disorder the spine &amp; cause fatigue</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>7</td>
<td>Radiation like in welding radioactive substance could be emitted as I perform my job function</td>
<td>2.4</td>
<td>MA</td>
</tr>
<tr>
<td>8</td>
<td>Radiation could cause cancer &amp; premature skin aging</td>
<td>2.8</td>
<td>HA</td>
</tr>
<tr>
<td>9</td>
<td>Carry gas bottles every day</td>
<td>2.2</td>
<td>MA</td>
</tr>
</tbody>
</table>

*H.A. = High Awareness, M.A. = Moderate Awareness, L.A. = Low Awareness

Table (2) this table shows that mean of scores of Workers Staff of kirkuk and tikrit city was high Awareness in all items

Table (3): Comparative about Awareness of gas station workers regarding Chemical Health Hazards at Kirkuk city and Tikrit city

<table>
<thead>
<tr>
<th>No</th>
<th>items</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Assess of awareness</td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>Working with chemical substances is part of my job function</td>
<td>2.3</td>
<td>MA</td>
</tr>
<tr>
<td>2</td>
<td>The substance are solvent, mist, fum, and gas</td>
<td>2.5</td>
<td>HA</td>
</tr>
<tr>
<td>3</td>
<td>The chemical / gases are flammable, poisonous &amp; corrosive</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>4</td>
<td>The hazardous chemicals are sometimes inhaled, ingested, injected and spill over my skin</td>
<td>2.0</td>
<td>MA</td>
</tr>
<tr>
<td>5</td>
<td>Eating where there are chemical is highly prohibited</td>
<td>1.8</td>
<td>MA</td>
</tr>
</tbody>
</table>
Cont... Table (3): comparative about Awareness of gas station workers regarding Chemical Health Hazards at Kirkuk city and tikrit city

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Assess of awareness</td>
</tr>
<tr>
<td>6</td>
<td>Chemical hazards are likely to affect ones health when they are exposed to them for a long period of time</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>7</td>
<td>Exposure to chemical hazards could cause reproductive disorder, cardiovascular diseases, respiratory disease, renal disease</td>
<td>2.0</td>
<td>MA</td>
</tr>
<tr>
<td>8</td>
<td>The health impact of chemical hazards could lead to loss of life</td>
<td>2.6</td>
<td>HA</td>
</tr>
</tbody>
</table>

*H.A.= High Awareness, M.A.=Moderate Awareness, L.A.=Low Awareness

Table (3) this table shows that mean of scores of Workers Staff of tikrit city was high Awareness in all items in comparative of kirkuk city which was was moderate Awareness

Table (4): comparative about Awareness of gas station workers regarding Mechanical Health Hazards at Kirkuk city and tikrit city

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Assess of awareness</td>
</tr>
<tr>
<td>1</td>
<td>Working with chemical substances is part of may job function</td>
<td>2.3</td>
<td>MA</td>
</tr>
<tr>
<td>2</td>
<td>The substance are solvent, mist, fum, and gas</td>
<td>2.5</td>
<td>HA</td>
</tr>
<tr>
<td>3</td>
<td>The chemical/gases are flammable, poisonous &amp; corrosive</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>4</td>
<td>The hazardous chemicals are sometimes inhaled, ingested, injected and spill over my skin</td>
<td>2.0</td>
<td>MA</td>
</tr>
<tr>
<td>5</td>
<td>Eating where there are chemical is highly prohibited</td>
<td>1.8</td>
<td>MA</td>
</tr>
<tr>
<td>6</td>
<td>Chemical hazards are likely to affect ones health when they are exposed to them for a long period of time</td>
<td>2.6</td>
<td>HA</td>
</tr>
<tr>
<td>7</td>
<td>Exposure to chemical hazards could cause reproductive disorder, cardiovascular diseases, respiratory disease, renal disease</td>
<td>2.0</td>
<td>MA</td>
</tr>
<tr>
<td>8</td>
<td>The health impact of chemical hazards could lead to loss of life</td>
<td>2.6</td>
<td>HA</td>
</tr>
</tbody>
</table>

*H.A.= High Awareness, M.A.=Moderate Awareness, L.A.=Low Awareness
Table (4) this table shows that mean of scores of Workers Staff of tikrit city was high Awareness in all items in comparative of kirkuk city which was was moderate Awareness

**Table (5): comparative about Awareness of gas station workers regarding Psychosocial Health Hazards at Kirkuk city and tikrit city**

<table>
<thead>
<tr>
<th>No</th>
<th>items</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Assess of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>awareness</td>
</tr>
<tr>
<td>1</td>
<td>My workload is very challenging</td>
<td>2.0</td>
<td>M.A.</td>
</tr>
<tr>
<td>2</td>
<td>I would like to be transferred to another unit /department</td>
<td>2.1</td>
<td>M.A.</td>
</tr>
<tr>
<td>3</td>
<td>I work in solation</td>
<td>2.1</td>
<td>M.A.</td>
</tr>
<tr>
<td>4</td>
<td>I am faced with some kind of aggression &amp; harassment in my place of work</td>
<td>1.2</td>
<td>L.A.</td>
</tr>
<tr>
<td>5</td>
<td>Psycho social hazards could cause hypertension, anxiety boredom</td>
<td>2.2</td>
<td>M.A.</td>
</tr>
</tbody>
</table>

*H.A.= High Awareness, M.A.=Moderate Awareness, L.A.=Low Awareness

Table (5) this table shows that mean of scores of Workers Staff of tikrit city was high Awareness in all items in comparative of kirkuk city which was was moderate and low Awareness

**Table (6): comparative about Awareness of gas station workers regarding Biological Health Hazards at Kirkuk city and tikrit city**

<table>
<thead>
<tr>
<th>No</th>
<th>items</th>
<th>Kirkuk city</th>
<th>Tikrit city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Assess of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>awareness</td>
</tr>
<tr>
<td>1</td>
<td>Microbes could be found in some substance I work with in my work station</td>
<td>1.6</td>
<td>L.A.</td>
</tr>
<tr>
<td>2</td>
<td>I general hazardous waste while working</td>
<td>2.2</td>
<td>M.A.</td>
</tr>
<tr>
<td>3</td>
<td>some of this hazardous waste could impact on the health of workers</td>
<td>2.1</td>
<td>M.A.</td>
</tr>
<tr>
<td>4</td>
<td>Biological hazards could cause Tuberculosis, pneumonitis</td>
<td>2.0</td>
<td>M.A.</td>
</tr>
<tr>
<td>5</td>
<td>proper environmental hygiene is lacking in my place of work</td>
<td>1.9</td>
<td>M.A.</td>
</tr>
</tbody>
</table>

*H.A.= High Awareness, M.A.=Moderate Awareness, L.A.=Low Awareness
Table (6) this table shows that mean of scores of Workers Staff of tikrit city was high and moderate Awareness in all items in comparative of kirkuk city which was was moderate Awareness

**Discussion of the Results**

The Results shows in Table (1) had revealed that an age group was high at (30-39) yrs in both kirkuk company gas filling and tikrit company gas filling and constitute 26.0% and 29.0 respectively

Explanation of this result because the system in our country most of the graduates students from the Institute of oil and gas direct work in the Kirkuk and tikrit gas company there for we find high percentage from the workers at age (30-39) yrs in both kirkuk and tikrit gas company

Our reslut is agreement with other study conducted by Achalu 2016 found that the staff of age group greater percent within (30-40 years) and constitute 30.0%

Also the result of the present study indicate The staff were male is high percentage in company gas filling / branch Kirkuk and branch tikrit ,and constitute 95.0% and 98.0% in both kirkuk and tikrit company respectively

Interpret of this result Because of the work at company need heavy work and dangerous and carrying heavy things there for we find great percentage from workers was male.

The current investigation is concurrence with Heymann (2016) discovered Sample included 90.5% (n=200) guys and 9.5% (n=21) females in Because of substantial work and perilous and conveying overwhelming things to be the most male laborers due to the working environment

Likewise the outcomes shows the high rate 78.0% and 82.0% were immediate contact to the gas from kirkuk and tikrit respectively

The decipher of this outcome identified with the kind of work at gas organization and the organization gas filling is more requirement for physically laborers there for we discover high rate were immediate contact.

With regard to the time of work per day the results of the present study show that the highest percentage is 78.0% and 73.0% were work (6-8) hours at kirkuk and tikrit respectively .the explanation of this result related to the system work at gas filling company need for long time working there for we find high percentage from workers work for at lest 6-8 hours

Table (2) Comparative about awareness of gas station workers regarding physical hazard at at Kirkuk city and Tikrit city the reult of this table shows that mean of scores of workers staff of kirkuk and tikrit city was high Awareness in all items

Physical dangers that influence wellbeing laborers incorporate introduction to commotion, vibration, ionizing and no ionizing radiation, and power. With the exception of very noisy commotion of a hazardous or effect nature where some measure of hearing misfortune and additionally auxiliary harm happens (acoustic injury), uproarious clamor at first uniform the sensitive hair cells in the inward ear causing a move in hearing limit. This is known as a transitory limit move (TTS) (6).

A basic test can be led by laborers to survey the impacts of word related introduction to clamor and its effect on hearing sharpness a few substances might be basically innocuous in certain structures, (for example, a square of metal, a bit of wood or granulated strong synthetic compounds) however might be perilous in another structure, (for example, fine residue particles or smoke that can be promptly breathed in or arrangements that might be sprinkled and promptly consumed through skin). This is additionally a significant thought in surveying dangers from physicochemical risks. The grouping of risky fixings is likewise a significant factor in the general hazard. Concentrates or unadulterated substances might be amazingly dangerous, while weaken arrangements of a similar concoction may not be risky at all. The extraordinary warmth of welding and starts can cause consumes. Eye wounds have come about because of contact with hot slag, metal chips, sparkles, and hot terminals. Furthermore, over the top presentation to warmth can bring about warmth stress or warmth stroke. Welders ought to know about the side effects, for example, exhaustion, unsteadiness, loss of hunger, sickness, stomach torment, and fractiousness. Ventilation, protecting, rest breaks, and remaining hydrated will ensure against heat

Table (3):comparative about Awareness of gas station workers regarding Chemical Health Hazards at
Kirkuk city and Tikrit city. This table shows that mean of scores of Workers Staff of Tikrit city was high Awareness in all items in comparative of Kirkuk city which was was moderate Awareness.

The explanation of this result related to safety measure at Kirkuk gas company more than Tikrit gas company.

Exposure standards are based on the airborne concentrations of individual substances that, according to current knowledge, should neither impair the health of, nor cause undue discomfort to, nearly all workers. They do not represent a fine dividing line between a healthy and unhealthy work environment. Chemicals with workplace exposure standards are listed in the *Workplace Exposure Standards for Airborne Contaminants*. These exposure standards are also available from the Hazardous Substances Information System (HSIS) on the Safe Work Australia website. The HSIS database contains additional information and guidance for many substances. Although exposure standards may also be listed in Section 8 of the SDS, you should always check the *Workplace Exposure Standards for Airborne Contaminants* Some substances give off distinctive odours which can alert workers to the presence of a hazardous chemical. For example, hydrogen cyanide has a smell of bitter almonds. However, not everyone can smell hydrogen cyanide and higher concentrations of hydrogen cyanide can also overload nasal receptors resulting in workers being unable to detect it. Hazardous chemicals can also have no odour. Thus, odour should not be relied on as a means of detecting the presence of hazardous chemical.

Table (4): comparative about Awareness of gas station workers regarding Mechanical Health Hazards at Kirkuk city and Tikrit city, this table shows that mean of scores of Workers Staff of Tikrit city was high Awareness in all items in comparative of Kirkuk city which was was moderate Awareness.

Interpret of this result refers to the types of work at Tikrit gas company more hard than Kirkuk gas company.

Table (5): comparative about Awareness of gas station workers regarding Psychosocial Health Hazards at Kirkuk city and Tikrit city, this table shows that mean of scores of Workers Staff of Tikrit city was high Awareness in all items in comparative of Kirkuk city which was was moderate and low Awareness.

Absence of inspiration and regard, prompting low degrees of efficiency. Factors, for example, quality client support, group attachment and group building are influenced because of representative truancy and absence of interest. Significant levels of representative turnover are additionally experienced. While trying to decrease representatives being exhausted and feeling less enthused while at work, it is significant that those at the top, for example, supervisors perceive the pressure and weights felt by representatives when they are given such a large number of duties. Administrators must devise approaches to draw out the best in representatives to propel them and lift their regard to guarantee an occupation all around done. This can be as straightforward as regarding representatives as a greater amount of a resource for the association by demonstrating gratefulness for their difficult work and commitment. Notwithstanding how extreme, solid and versatile you think you are, toward the day’s end, we are for the most part people, and as such bosses should avoid seeing them as machines. There’s a cutoff to the amount we can propel ourselves truly, yet our enthusiastic perseverance can be driven much further. It’s essential to know your cutoff points by methods for working SMART. You’re nothing but bad to anybody, in particular yourself in case you’re not in top mental and physical condition.

Table (6): comparative about Awareness of gas station workers regarding Biological Health Hazards at Kirkuk city and Tikrit city, this table shows that mean of scores of Workers Staff of Tikrit city was high and moderate Awareness in all items in comparative of Kirkuk city which was was moderate Awareness.

Eduardodias health (2016) report that the Gasoline, a straightforward, fluid got from oil contains two fundamental synthetics: benzene (C₆H₆) and other known cancer-causing agents. Cancer-causing agents don’t straightforwardly influence DNA, however lead to malignant growth in different manners. For instance, they may make cells isolate at a quicker than ordinary rate, which could build the odds of changes to DNA. Note that not all cancer-causing agents bring about malignancy. Numerous variables must be contemplated length and power of the exposure.

Wluk (2018) report that the natural and irresistible
specialists might be sent to an individual through inward breath, infusion, or by skin contact. Sources incorporate patients, asymptomatic transporters, or vectors, for example, rodents, cockroaches, and mosquitoes. The quantity of living beings in nature, combined with their destructiveness and an individual’s protection from them, decide if the individual will get the infection or not. A contamination control program ought to characterize the fundamental strategies, methodology, and practices so as to limit the danger of malady event and transmission at a human services office. This necessitates laborers be counseled and that the exertion is bolstered by the entirety of the executives and staff(11).

Conclusions

The demographic data for both kirkuk and Tikrit city the result aso shows that the age group (20-29 years) represents a high percentage in the Kirkuk gas and Tikrit city and represent (40.0%) and (38.0%) respectively. With regard to the gender the results shows high percentage from gender were male in kirkuk and tikrit and represent (95.0%) and (98.0%) respectively. also the result shows high percentage from workers was worked at direct department contact in kirkuk and tikrit and represent (78.0%) and (82.0%) respectively. The physical hazard were same level in both Tikrit and Kirkuk workers. The results Shows the psychological, biological and psychological hazards most common at Tikrit workers than Kirkuk.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Adult Nursing and all experiments were carried out in accordance with approved guidelines.

References

Non COVID Symptoms and its Impact on Quality of Life among the Professionals Residing at Pune City

Sharadha Ramesh¹, Poonam Yadav²

¹Director cum Professor, Symbiosis College of Nursing, Symbiosis International University, Pune, Maharashtra, India, ²Tutor, Symbiosis College of Nursing, Symbiosis International University, Pune, Maharashtra, India

Abstract

Introduction: COVID-19 has made all of us understandably too much cautious to visit the physician’s. We have rescheduled the appointments and trying to manage our care viva virtual visits, Also trying to stay safe by restricting movements. It’s important to keep taking precautions like handwashing, avoid touching face area and also wearing the masks in public. Hence it’s very important for all of us to know when to seek medical help.

Objectives of the study: 1. To assess the Non COVID Symptoms among working professionals in Pune City 2. To assess the impact of Non Covid symptoms on quality of life among working professionals in Pune City 3. To associate the selected Demographic variable with the Non COVID Symptoms among working professionals in Pune City

Methodology: Survey study was conducted among 265 working professionals in Pune City. Non probability convenience sampling technique was used to select the samples. Informed consent was obtained from all participants, and made them aware that the information gathered is used only for research purpose. Structured questionnaires were prepared to elicit information from the participants.

Result: The study result says that are various Non Covid symptoms which the general population is facing in the current scenario which are being identified in the study result and also the Non Covid symptoms are being associated with the various selected demographic variable the result pertaining to that shows that there is significant association between working profession and monthly family income with Non COVID Symptoms as the P value is less than 0.05. In this current situation the investigator wanted to recommended further studies to find out the various other Non covid symptoms faced by the general population an should be paid attention and treated on time.

Key Words: Non COVID, Symptoms, Impact, Quality of life, Professionals.

Introduction

COVID-19 has made us prudent to visit the hospital in any means. Individuals are trying hard to manage their care at home by avoiding visits to the clinics so as to avoid getting exposed to Covid virus. This has made everyone to take measures that promotes health and wellbeing of ourselves. Certain hygienic measures needs to be taken by each and every one to avoid falling prey to Covid. This includes washing hands thoroughly for 20 seconds, maintaining respiratory hygiene, following social distancing practice. Recently all are following the guidelines given by WHO but it is important for all of us to know when to seek medical help. It is necessary for us to know and understand the non covid symptoms so as to deal with it optimist. The non covid symptoms include headache, eye irritation, fatigue, loss of tired, irregularity of bowel, weight alteration, mood swings, fear, skin irritation, body ache. (1,2,3)

Statement of the Problem

An exploratory study to assess the Non COVID Symptoms its impact on quality of life among the professionals residing at Pune City
Objectives:

1. To assess the Non COVID Symptoms among working professionals in Pune City
2. To assess the impact of Non Covid symptoms on quality of life among working professionals in Pune City
3. To associate the selected Demographic variable with the Non COVID Symptoms among working professionals in Pune City

Methodology

RESEARCH APPROACH

Quantitative research approach was adopted for this study to accomplish the objectives of the study.

RESEARCH DESIGN

In this study, Non-experimental Exploratory survey design was used.

VARIABLES

Independent Variables:

The independent variable for the study was Non COVID Symptoms.

Dependent Variables:

The dependent variables of the study was Quality of life.

SETTING OF THE STUDY

The investigator conducted the study in urban area of Pune City.

POPULATION OF THE STUDY

SAMPLE AND SAMPLE SIZE

In the present study, the professionals residing in the selected area of Pune City. Who fulfilled the inclusive criteria were the samples.

SAMPLE SIZE

The sample size was 265.

SAMPLING TECHNIQUES

Non probability convenience sampling technique was used to select the samples.

DEVELOPMENT AND DESCRIPTION OF THE TOOL

The instrument was developed and complied by the investigator with the guidance of experts and review of literature. The data collection questionnaire used in the present study had the following components:

Section A: Assessment of background variables

- Demographic variables of the mother:

This included study participants age, religion, type of family, monthly income in rupees, educational status

Section B: Structured Question on Non Covid Symptoms.

Section C: Structured Checklist on Impact on Quality of Life.

CONTENT VALIDITY

Content validity of instrument was done by the panel of experts in the fields of Nursing Research and Statistics. The experts’ suggestions were incorporated in designing the final tool for this study.

RELIABILITY

Reliability of the tool was measured using Karl’s Pearson coefficient of correlation’ test retest method for Non Covid symptoms structured questionnaires and inter rater method for perceived practice.

The reliability r value were 0.78. These values were very high thus making it a reliable tool for assessing the “Non COVID Symptoms and its impact on quality of life among the professionals residing at Pune City”.
DATA ANALYSIS AND INTERPRETATION

DEMOGRAPHIC DATA

Table 1: Description of Demographic data in terms of Frequency and percentage distribution

\[ N=265 \]

<table>
<thead>
<tr>
<th>SR</th>
<th>Demographic variable</th>
<th>Freq</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 22 years</td>
<td>70</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>22 – 24 years</td>
<td>85</td>
<td>32.1</td>
<td></td>
</tr>
<tr>
<td>24 – 26 years</td>
<td>50</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>26 and above</td>
<td>60</td>
<td>22.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>112</td>
<td>42.3</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>153</td>
<td>57.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear family</td>
<td>160</td>
<td>60.4</td>
<td></td>
</tr>
<tr>
<td>Joint family</td>
<td>96</td>
<td>36.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Profession</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government</td>
<td>90</td>
<td>34.0</td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>66</td>
<td>24.9</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>99</td>
<td>38.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Religion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hindu</td>
<td>155</td>
<td>60.5</td>
<td></td>
</tr>
<tr>
<td>Muslim</td>
<td>51</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Sikh</td>
<td>20</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Christian</td>
<td>10</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Monthly family income in rupees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-30 k/Month</td>
<td>60</td>
<td>23.4</td>
<td></td>
</tr>
<tr>
<td>above 30 K/month</td>
<td>40</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>Below 30 K/Month</td>
<td>155</td>
<td>60.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Educational Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graduate or post graduate</td>
<td>50</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>Intermediate or post high school diploma</td>
<td>70</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td>High school certificate</td>
<td>80</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>Middle school certificate</td>
<td>25</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>Primary school certificate</td>
<td>30</td>
<td>11.7</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Description of Non COVID Symptoms among the professionals residing at Pune City

N= 265

<table>
<thead>
<tr>
<th>Item Freq</th>
<th>Never Happens to me</th>
<th>Sometimes Happens to me</th>
<th>Always Happens to me</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Freq</td>
<td>%</td>
<td>Freq</td>
</tr>
<tr>
<td>1 Do you feel headache on regular basis</td>
<td>40</td>
<td>15.09</td>
<td>85</td>
<td>32.08</td>
</tr>
<tr>
<td>2 Do your eyes had irritation or discharge</td>
<td>30</td>
<td>11.32</td>
<td>105</td>
<td>39.62</td>
</tr>
<tr>
<td>3 Do you feel fatigue and tired</td>
<td>30</td>
<td>11.32</td>
<td>115</td>
<td>43.40</td>
</tr>
<tr>
<td>4 Do you feel loss of appetite</td>
<td>20</td>
<td>7.55</td>
<td>115</td>
<td>43.40</td>
</tr>
<tr>
<td>5 Do have a complain of irregularity of bowel</td>
<td>15</td>
<td>5.66</td>
<td>165</td>
<td>62.26</td>
</tr>
<tr>
<td>6 Do you have sleep disturbance</td>
<td>25</td>
<td>9.43</td>
<td>130</td>
<td>49.06</td>
</tr>
<tr>
<td>7 Did you noticed and recorded weight alteration</td>
<td>35</td>
<td>13.21</td>
<td>100</td>
<td>37.74</td>
</tr>
<tr>
<td>8 Do you get mood swings</td>
<td>40</td>
<td>15.09</td>
<td>85</td>
<td>32.08</td>
</tr>
<tr>
<td>9 Do you feel fear of uncertainty</td>
<td>26</td>
<td>9.81</td>
<td>139</td>
<td>52.45</td>
</tr>
<tr>
<td>10 Have of experienced panic</td>
<td>29</td>
<td>10.94</td>
<td>159</td>
<td>60.00</td>
</tr>
<tr>
<td>11 Did you notice any skin irritation or changes?</td>
<td>89</td>
<td>33.58</td>
<td>126</td>
<td>47.55</td>
</tr>
<tr>
<td>12 Did you had complain of body ache</td>
<td>16</td>
<td>6.04</td>
<td>156</td>
<td>58.87</td>
</tr>
</tbody>
</table>

Above table no 2 Describes the Non Covid symptoms in selected professionals of Pune City.

The various symptoms which were identified in the current study are headache, eyes irritation, fatigue and tired, loss of appetite, bowel irregularity, sleep disturbance, weight alteration, mood swings, fear of uncertainty, panic, skin irritation, body ache. The results regarding headache includes 52.83% professionals experience headache more frequently whereas 32.08% professionals experience it less frequently and 15.09% professionals never experienced headache. The eyes irritation and discharge results suggests that 49.057% experience it more frequently whereas 39.62% professionals experience it less frequently and 11.32% professionals have never experienced it. The results regarding feeling tired and fatigue includes 45.283% professionals experience it more frequently whereas 43.40% professionals experience it less frequently and 7.55% professionals have never experienced tiredness and fatigue. The loss of appetite results include 49.057% professionals experience it more frequently whereas 43.40% professionals experience it less frequently and 7.55% professionals have never experienced it. The results regarding the irregularity of bowel include
33.962% professionals experience it more frequently whereas 62.26% professionals experience bowel irregularity less frequently and 5.66% professionals have never experienced bowel irregularity. The sleep disturbance results include 41.509% professionals experience sleep disturbance more frequently; whereas 49.06% professionals experience it less frequently and 9.43% professionals have never experienced sleep disturbances. The weight alteration result includes 49.057% professionals experience it more frequently; whereas 37.74% professionals experience it less frequently and 13.21% professionals have never experienced it. The mood swing results exhibits 52.83% professionals experience mood swings more frequently; whereas 32.08% professionals experience mood swings less frequently and 15.09% never experienced mood swings. The results regarding feeling fear of uncertainty revealed that 37.736% professionals experience it more frequently whereas 52.45% professionals experience it less frequently and very few 9.81% have never experienced fear of uncertainty. The panic experience includes 30.189% get panic more frequently; whereas 60% professionals experience panic less frequently and 10.94% professionals never feel panic. The Skin irritation changes results interpret that 18.868% professionals have more frequently experienced skin irritation, majority 47.55% professionals have experienced it less frequently whereas 33.58% professionals have never experienced skin irritation and changes. The result regarding the feel of bodyache on regular basis showed that 52.83% of the professionals had more frequently; whereas 58.87% of the professionals had less frequently experienced bodyache and 6.04% professionals have never experienced bodyache.

Table 3. Description of impact of the Non COVID Symptoms on Quality of life

No=265

<table>
<thead>
<tr>
<th>QOL</th>
<th>Never Happens to me</th>
<th>Sometimes Happens to me</th>
<th>Always happens to me</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very Good</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Freq</td>
<td>%</td>
<td>Freq</td>
<td>%</td>
</tr>
<tr>
<td>0-7</td>
<td>08 to 14</td>
<td>15 to 21</td>
<td></td>
</tr>
<tr>
<td>1 Physical</td>
<td>60</td>
<td>23.44</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QOL</td>
<td>Never Happens to me</td>
<td>Sometimes Happens to me</td>
<td>Always happens to me</td>
</tr>
<tr>
<td></td>
<td>Very Good</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Freq</td>
<td>%</td>
<td>Freq</td>
<td>%</td>
</tr>
<tr>
<td>0-9</td>
<td>10 to 18</td>
<td>19 to 27</td>
<td></td>
</tr>
<tr>
<td>2 Mental/Psychology</td>
<td>19</td>
<td>7.42188</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QOL</td>
<td>Never Happens to me</td>
<td>Sometimes Happens to me</td>
<td>Always happens to me</td>
</tr>
<tr>
<td></td>
<td>Very Good</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Freq</td>
<td>%</td>
<td>Freq</td>
<td>%</td>
</tr>
<tr>
<td>0-6</td>
<td>7 to 12</td>
<td>13-18</td>
<td></td>
</tr>
<tr>
<td>3 Social</td>
<td>56</td>
<td>21.875</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Considering the physical factor, 46.88% professionals more frequently experience that the non covid symptoms have affected their physical wellbeing whereas 29.69% professional has experienced less frequent effect of non covid symptoms on their physical wellbeing and 23.44% professionals never experienced any impact on their physical wellbeing.

The mental/psychological factor suggests, 58.5938% professionals more frequently experience that the non covid symptoms have affected their mental wellbeing whereas 33.20313% professional has experienced less frequent effect of non covid symptoms on their mental wellbeing and 7.42188% professionals never experienced any impact on their mental wellbeing.

The social factor reveals, 50.7813% professionals more frequently experience that the non covid symptoms have affected their social wellbeing whereas 27.3438% professional has experienced less frequent effect of non covid symptoms on their social wellbeing and 21.875% professionals never experienced any impact on their social wellbeing.

The financial factor displays, 43.3594% professionals more frequently experience that the non covid symptoms have affected their financial wellbeing whereas 15.625% professional has experienced less frequent effect of non covid symptoms on their financial wellbeing and 10.1563% professionals never experienced any impact on their financial wellbeing.

The level of independence mounts that, 74.2188% professionals more frequently experience that the non covid symptoms have affected their level of independence whereas 27.3438% professional has experienced less frequent effect of non covid symptoms on their level of independence and 29.2969% professionals never experienced any impact on their level of independence.

Considering the environmental factor, 31.25% professionals more frequently experience that the non covid symptoms have affected their level of independence whereas 39.0625% professional has experienced less
frequent effect of non covid symptoms on their level of independence and 29.6875% professionals never experienced any impact on their level of independence.

Description of the association of selected demographic variables with Non COVID Symptoms among the selected professionals.

In proceeding To find out the association of demographic data with Non COVID Symptoms data was analyzed and found that there is significant association between working profession and monthly family income with Non COVID Symptoms as the P value is less than 0.05.

Conclusion

The above study result says that are various Non Covid symptoms which the general population is facing in the current scenario which are being identified in the study result and also the Non Covid symptoms are being associated with the various selected demographic variable the result pertaining to that shows that there is significant association between working profession and monthly family income with Non COVID Symptoms as the P value is less than 0.05. In this current situation the investigator wanted to recommended further studies to find out the various other Non covid symptoms faced by the general population an should be paid attention and treated on time.

Implications

The findings of the study has implications in different branches of nursing profession, i.e. ursing practice, nursing service, nursing education, nursing administration and nursing research.

Nursing practice.

- The nurse role is to care for the patients according to their health condition to improve their quality of life.

- The nurse practitioners can utilize the assessment toll and identify the symptoms are related to COVID or Non Covid in the client to improve their quality of life.

- The nurse can educate the population to reduce their anxiety raised due to Non Covid symptoms and to improve their quality of life.

Nursing education

- Conference, workshops and seminars can be planned and executed for nurses to impart update their knowledge and positive practice towards care of Non Covid Symptoms and quality of life.

- Nursing educator to update their knowledge and skills of providing care for patients with Non Covid symptoms and quality of life.

Nursing administration

- Nursing personal should be prepared to take a leadership role in educating the population around her to take care and differentiate the Covid, Non Covid Symptoms and points to improve quality of life.

- The administrator can encourage the nurse for conducting research in various aspects regarding the Non Covid Symptoms and quality of life in the general population.

- The administrator can organize conference, workshop and seminar for nurses regarding the Non Covid Symptoms and quality of life in the general population.

- The administrator should support the staff to conduct programme on various Non Covid Symptoms and quality of life.

Nursing Research

- There should be more scope for the research in this area to improve the care for clients having Non Covid Symptoms and to improve their quality of life.

- Knowledge on importance quality of life and Non Covid symptoms is important for the general population to know. There is a need for extensive research regarding education on Non Covid Symptoms and to improve their quality of life.

- The study will be useful for further reference.

- The results of the study encourage the nursing personnel’s for conducting research in various aspects regarding importance to know various Non Covid Symptoms and quality of life.

Recommendations

The study recommends the following...
• A similar study may be conducted with large number of sample and population in various settings.

• A similar study may be conducted with large number of sample and population in various settings.

• A comparative study can be conducted between rural and urban population.

• A true experimental study with experimental and control group can be conducted.

Conflict of Interest – Nil

Source of Funding - Authors have no financial support to this project. The study was fully funded by authors.

Ethical Clearance – Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the professional included in the study. Informed the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential

References


Impact of Years’ Experience upon Nurses’ Knowledge and Practice concerning Infection Control at Critical Care Units in Baghdad City

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¹Ph.D. (University of Baghdad-College of Nursing-Adult Nursing Department), ²M.Sc. (University of Baghdad-College of Nursing-Adult Nursing Department)

Abstract

Introduction: Infection control or hospital-acquired infections are the major concern of the health care system and agencies. Critical care nurses are on the first-line contact with the patients, so on, they are most vulnerable to acquired infections. It is really important to regularly check their knowledge and practices concerning infection control. Objectives: The study aims to identify the impact of years’ experience on nurses’ knowledge and practices concerning infection control in three hospitals and center (Baghdad teaching hospital, Ibn Al-Nafees hospital, and Ibn al-Bitar center) Methodology: Cross-sectional study was conducted, the study starting from 4th of July 2020 to 13th of November 2020. Non-probability (purposive) sample was used to select 110 nurses who work at critical care units in three hospitals and center (Baghdad teaching hospital, Ibn Al-Nafees hospital, and Ibn al-Bitar center). The years of experience should no less than one year. The instrument was composed of two parts. Firstly, covers the nurses’ demographic characteristics. Secondly, included self-report questions about nurses’ knowledge and an evaluation sheet concerning nurses’ practices of infection control with 24 questions for knowledge and 12 questions for practices checklist. Results: The study revealed that the nurses’ knowledge toward infection control record 38%, 41%, and 21% for low, moderate, and high knowledge respectively. In comparison, nurses practices record 47%, 42%, and 11% for low, moderate, and high practices respectively. Most of the study sample 62% was females while 38 % of the subject was males. The age group (20-29) takes the highest percentage (38%). In addition, about (27.3%) of the nurses who works in critical care units have 1-5 years of experience. Significant statistical associations were found between nurses knowledge and practice from one hand and years’ experience on the other hands p ≤ 0.05. Recommendation: Based on this finding, the researcher recommended further studies involve more nurses and other health professionals regarding infection control and highlight the importance of infection control to nurses with (1-5) years’ experience by symposium or teaching programs.

Keywords: Impact, Years’ Experience, Nurses’ Knowledge & Practice, Infection Control, Critical Care Units

Introduction

Hospital-acquired infections are significantly high, especially in critical care units. Both nurses and patients are vulnerable to get those infections, patients in critical care units have low immunity as a result of their health status and can easily be infected with viruses and bacteria [1, 2, 3]. In comparison, nurses are in direct contact with patients for a long time [4, 5]. Nurses’ knowledge and practices about precautions and infection prevention strategies are the cornerstones to minimize the burden of hospital-acquired infections [6]. Accordingly, Information about nosocomial diseases is imperative to improve infection control strategies and to create successful preventive and therapeutic methodologies which, thus, will help us in diminishing the prevalence, and mortality rate [7, 8]. The infection control is a key part of training for all health services personnel, for their health as well as to minimize nosocomial infection and along these lines improve nurses and patients safety [9, 10].
Methodology and Materials

Design of the study:

A cross-sectional research design was conducted from the period 4th of July 2020 to 13th of November 2020 to find out the impact of years’ experience on nurses knowledge and practices concerning infection control. Hospitals and center (Baghdad teaching hospital, Ibn Al-Nafees hospital, and Ibn al-Bitar center) in Baghdad city were included. Non probability (purposive) sample was used to select 110 nurses who worked in critical care units and have at least one year experience.

Tools of the study:

It was consisted of two parts, first part: composed of nurses’ demographic characteristics include age, gender, and years of experience in the critical care units. Second part: consist of (24) self-reported questions for nurses’ knowledge and (12) questions as a checklist evaluation for practices concerning infection control.

Validity and Reliability

The content validity of the knowledge and practices sheet was checked by ten experts in College of Nursing / University of Baghdad with different specialty and modifications done accordingly. Reliability was done to determine the stability of the questionnaire by using test-retest method (correlation coefficient 0.89).

Results

Figure 1: Distribution of Nurses’ Knowledge and Practices
Table 1: Distribution of Socio-Demographical Characteristics.

<table>
<thead>
<tr>
<th>Gender</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Female</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>30-39</td>
<td>30</td>
<td>27.3</td>
</tr>
<tr>
<td>40-49</td>
<td>20</td>
<td>18.3</td>
</tr>
<tr>
<td>50-59</td>
<td>18</td>
<td>16.4</td>
</tr>
<tr>
<td>Years of experience in critical care unit</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>1 – 5</td>
<td>30</td>
<td>27.3</td>
</tr>
<tr>
<td>6 – 10</td>
<td>15</td>
<td>13.6</td>
</tr>
<tr>
<td>11 – 15</td>
<td>17</td>
<td>15.4</td>
</tr>
<tr>
<td>16 – 20</td>
<td>20</td>
<td>18.3</td>
</tr>
<tr>
<td>21-25</td>
<td>12</td>
<td>10.9</td>
</tr>
<tr>
<td>More than 25 years</td>
<td>16</td>
<td>14.5</td>
</tr>
</tbody>
</table>

This table indicated that more than half (62%) of the study sample were females with (38%) at age group (20-29 years) and (27.3%) have (1-5) years’ experience.

Table 2: Association of years’ experience with Nurses knowledge

<table>
<thead>
<tr>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurses Knowledge</td>
</tr>
<tr>
<td>Sum of Squares</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

df: degree of freedom, F: frequency, sig: significant

This table demonstrates that there is a statistical significant association between years’ experience and nurses’ knowledge (p value ≤ 0.05).
Table 3: Association of years’ experience with Nurses Practice

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>389.942</td>
<td>4</td>
<td>97.486</td>
<td>2.425</td>
<td>0.053</td>
</tr>
<tr>
<td>Within Groups</td>
<td>4220.612</td>
<td>105</td>
<td>40.196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4610.555</td>
<td>109</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df: degree of freedom, F: frequency, sig: significant

Table (3) reveals significant statistical association between years’ experience and nurses’ practices in critical care units concerning infection control.

**Discussion**

Results in figure (1) shows that the moderate level of knowledge takes highest percentage (43%) concerning infection control while (47%) of the nurses records the high level practices. Most of the study (62%) sample was females, and the age group (20-29 years) takes the highest percentage (38%). The years’ experience (1-5) years records (27.3%) of the study sample.

This finding approximately similar with the study in Baghdad Teaching Hospital done by Abdel-latif, 56.6 were females, and 43.3% of them at age 20-29 years old, it is agree with the results of at eight hospitals in Saudia Arabia. Another study has been found that females were more Informationable than males toward infection control [11, 12].

The study results demonstrate that years’ of experience associate with nurses’ knowledge. In addition, there is a statistical significant association with nurses’ practices, the nurses have affected their compliances to standard precautions, thus more years of employment lead to more experiences which finally influences on nurses’ practices. This result agrees with study done by Eskander who find that there is a significant association between the Nurses’ Information and the years of employment [13]. Hassan, A. and Fatima were indicating positive relation between the skills and the years of experience as a nurse.

**Conclusions**

According to the results, the study conclude that years’ experience associate with the nurses knowledge and practices toward infection control in critical care units, the more years’ experience lead to more knowledge and skills toward infection control.

**Acknowledgements**

The authors feel privileged to thanks College of Nursing/ University of Baghdad for their cooperation to complete the official request for conducting the research.

**Conflict of Interest**

There are no conflict of interest concerning this research and the manuscript has not been submitted to another journal or publishing venue.

**Financial Resources**

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

**Informed Consent**

Before filling the questionnaire, all participants were asked if they agree or disagree to participate in the research.
Ethical Clearance

All experimental protocols were approved under the Faculty of Dentistry, University of Babylon, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Detection of Biochemical Causes the Diabetic Retinopathy in Diabetes (Type II) Patients in Al-Muthanna Province

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¹M.Sc., ²Prof., Dr., Department of Chemistry, College of Science, Al-Muthanna University, Iraq

Abstract

Background: Accumulating evidence indicates that oxidative stress, imbalance between reactive oxygen species production and antioxidant scavenging that may play a role in the etiology of type 2 diabetes mellitus and occurrence diabetic retinopathy as a result from increasing activity of free radicals and accumulation of lipid peroxidation products.

Aim: To investigate the association between glycemic control, lipid peroxidation and antioxidant status.

Methodology: A descriptive cross-sectional study on non-probability was conducted (sample collection) of (232) type 2 diabetes mellitus patients. Plain tubes, the tools and instruments that were used to collect data.

Results: The results of the study revealed that dyslipidemia and hypertension were found to be more prevalent in the diabetic retinopathy subjects than the regular diabetic subjects. Plasma antioxidant levels were higher in the diabetic subjects than the diabetic retinopathy subjects while malondialdehyde levels were found to be higher in the diabetic retinopathy subjects.

Conclusion: Duration of diabetes has very important effect on both MDA and uric acid levels for diabetic patients with/without retinopathy. Oxidative parameters value not influence by residence as well as body mass index not has any effect on uric acid level.

Keywords: Antioxidant Status, Oxidative Stress, Diabetic Retinopathy, Lipid Profile.

Introduction

Diabetic retinopathy (DR) is the most common disease that caused by micro-vascular complication of diabetes, occurs in both T1, T2 DM and is secondary to prolonged uncontrolled hyperglycemia and other risk factors. In adolescents and young adults with type 1 diabetes, over 80% have some form of DR when the duration of diabetes is over 15 years. It is estimated that 90% of the blindness due to DR is preventable (1) (2). Long term of high blood glucose levels in uncontrolled diabetes can be the cause of glucose auto-oxidation, nonenzymatic protein glycation and activation of the polyol pathway with increase oxidative stress.

The global number of individuals with diabetes in 2000 was estimated to be 171 million (2.8% of the world’s population). The number of people with DM worldwide is projected to increase to 366 million by 2030, 298 million of whom will live in developing countries (3) (4). There is a relationship between chronic hyperglycemia and long-term complications in diabetes. The long-term effects of diabetes include progressive evolution of micro-vascular complications, particularly in the eye and the kidney, and an increased frequency of macro-vascular disease such as peripheral vascular and coronary heart disease (3) (5). Complications of micro-vascular and macro-vascular are the leading cause of
morbidity and mortality in diabetics. However, diabetic patients often die from macro-vascular disease and main mortality is the coronary heart disease (CHD). Long-term complications of DM include retinopathy with potential vision loss (6) (7) (8) (9).

Diabetic retinopathy (DR) is the most common disease that caused by micro-vascular complication of diabetes, occurs in both T1, T2 DM and is secondary to prolonged uncontrolled hyperglycemia and other risk factors. In adolescents and young adults with type 1 diabetes, over 80% have some form of DR when the duration of diabetes is over 15 years. It is estimated that 90% of the blindness due to DR is preventable (1) (2). Long term of high blood glucose levels in uncontrolled diabetes can be the cause of glucose auto-oxidation, nonenzymatic protein glycation and activation of the polyol pathway with increase oxidative stress.

Oxidative stress is the result of an imbalance in the pro-oxidant/antioxidant ratio in favor of the former, potentially leading to form macromolecules and dysfunction in the cell. Oxidative stress is the result of an imbalance in the pro-oxidant/antioxidant ratio in favor of the former, potentially leading to form macromolecules and dysfunction in the cell (10). Improved oxidative stress contributes to the damage of pancreatic β-cell progressively due to glucose toxicity, which leads to severe weakness of glucose-stimulated insulin secretion (11).

In normal physiological conditions, there is a balance in the generation of reactive oxygen and nitrogen species (ROS, RNS) and antioxidant defense system to protects organisms against ROS, RNS toxicity. In diabetes, imbalance in the pro-oxidant/antioxidant can damage cellular macromolecules, leading to protein modification and lipid peroxidation (12). Lipid peroxidation is an autocatalytic free radical mediated destructive process where poly unsaturated fatty acids in cell membranes degraded to form lipid hydro peroxides. Through lipid peroxide products such as conjugated dienes and malondialdehyde (MDA) are increased in patients with T2DM. MDA is produced as a relatively stable end product from the oxidative degradation of polyunsaturated fatty acids (PUFA), and this free radical-driven lipid peroxidation has been causatively implicated in the aging process atherosclerosis, Alzheimer’s disease, and cancer (13) (14). Serum MDA used as a bio-marker of lipid peroxidation and served as an indicator of free radical damage (15).

The study of the oxidative stress status may be the knowledge base for understanding of the pathogenic mechanisms of complications in diabetes and may have important implications for antioxidant supplements in order to slow progress, choose optimal treatments and prevent complications and their consequences.

**Materials and Methods**

232 subjects as three groups, 58 patients (24 male and 34 female) have diabetic retinopathy, 78 patients (26 male and 52 female) have type 2 diabetes mellitus without retinopathy cases and 96 subjects (52 male and 44 female) as healthy control groups. For the purpose of this study, 232 subjects and age and gender-matched control subjects were recruited. Plain tubes, the tools and instruments that were used in the study.

**Metabolic biochemical markers for investigation**

1. Estimation of fasting plasma sugar by glucose oxidase/peroxidase method Trinder’s method.
2. Measurement of total cholesterol by Cholesterol esterase peroxidase or CHOD-POD method
3. Measurement of triglycerides by Lipoprotein lipase, Glycerol phosphate oxidase and Peroxidase. The method for the analysis is a modification of that of Trinder.
4. Measurement of malondialdehyde; MDA is determined as Thiobarbituric acid reactive substances.
5. Measurement of superoxide dismutase; Superoxide dismutase was measured by the method of Kuthan H, et al., (1986).
6. Measurement of total antioxidant capacity; Measures total antioxidant capacity in which Cu$$^{2+}$$ is reduced by antioxidant to Cu$^{+}$. The resulting Cu$^{+}$ specifically forms a colored complex with a dye reagent. The color intensity at 570 nm is proportional to TAC in the sample.

**Statistical Analysis**

Completely randomized design (CRD) was used
for this study in the analysis of variance for the means of studied parameters (sugar, lipid, oxidative stress, and antioxidants parameters) by using one-way ANOVA test, t-test, and Dennett’s test at a 5% level of significance. Moreover, data were processed and analysis by using statistical program social science (SPSS 22) and the results were expressed as Mean ± SD.

Results and Discussion

The study showed that T2DM and DR subjects had significantly higher FPS and HbA1c levels compared with control subjects according to reports of the American Diabetes Association (ADA) and the World Health Organization (WHO). Furthermore, increased HbA1c level was observed in DR more than T2DM. The results of the study under hand go along with study entitled (Total Antioxidant Status in Type 2 Diabetic Patients in Palestine) that carried out in Palestine found that there is a significant relationship between HbA1c and different group at ($\square = 0.002$) ($^{16}$). Also, in other study that carried out in African stated that the FPG and HbA1c levels were respectively 2.05 and 2.32 times higher in the group of patients with diabetes and complications compared to those of healthy persons ($^{17}$). HbA1c is a product of non-enzymatic addition of glucose to hemoglobin and the rate of this conjugation is directly proportional to the level of blood glucose. So, the high % of HbA1c level observed in our study denotes that the type 2 diabetic subjects are under poor glycemic control.

Figure (1): Comparison of HbA1c and FBS means among study groups.
This figure shows that significantly high levels of total cholesterol, triglyceride, LDL and VLDL in patients with T2DM than control. However, no significant difference in the level of HDL were identified between the three groups. These findings not harmonizing with study that conducted in Turkey stated that mean total cholesterol, triglyceride, LDL, HDL and VLDL levels were not significantly different between the groups ( =0.693, =0.774, =0.644, = 0.910 and =0.967 respectively (18)). However, it is going along with study that carried out in South Africa found that serum triglyceride (TG) ( = 0.004) and high-density lipoprotein cholesterol (HDL-C) ( = 0.007) showed significant differences among group (19). Moreover, study the proportions of lipid profile disorders were higher in DM patients compared to apparently healthy controls Moreover, study the proportions of lipid profile disorders were higher in DM patients compared to apparently healthy controls (20) (21).

Table (1) : Comparison of oxidative stress parameters means

<table>
<thead>
<tr>
<th>Oxidative Parameters</th>
<th>Control Mean±SD</th>
<th>DM Mean±SD</th>
<th>Retinopathy Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid mg/dl</td>
<td>4.5±1.5</td>
<td>4.9±1.3</td>
<td>4.7±1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a- &lt;0.0001*</td>
<td>b- 0.064</td>
<td>c- &lt;0.0001*</td>
<td></td>
</tr>
<tr>
<td>MDA µM</td>
<td>2.3±0.7</td>
<td>4.9±0.5</td>
<td>5.2±0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a- &lt;0.0001*</td>
<td>b- &lt;0.0001*</td>
<td>c- &lt;0.0001*</td>
<td></td>
</tr>
<tr>
<td>SOD U/ml</td>
<td>7.8±1.3</td>
<td>4.3±0.9</td>
<td>3.5±1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a- &lt;0.0001*</td>
<td>b- &lt;0.0001*</td>
<td>c- &lt;0.0001*</td>
<td></td>
</tr>
<tr>
<td>TAC mM</td>
<td>1.3±0.6</td>
<td>1±0.3</td>
<td>0.7±0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a- &lt;0.0001*</td>
<td>b- &lt;0.0001*</td>
<td>c- &lt;0.0001*</td>
<td></td>
</tr>
</tbody>
</table>
The result of current study goes along with study that conducted in Nigeria to investigate some oxidative stress related parameters in T2DM. Who found that significant differences of oxidative stress between patients and control groups (22). While, in regarding to uric acid the current study shows no significant difference in the level of uric acid between T2DP and healthy controls, and that inconsistent with study that carried out in India found that there is a significant difference between uric acid and three different group of study at $P<0.001$ (23). Glucose intake may affect both the production and excretion of uric acid by several mechanisms. An increased flux of glucose is thought to increase purine generation, while increased anaerobic glycolysis generates increased circulating lactate which leads to a reduction in renal excretion of uric acid (24).

Moreover, in other study that carried out in Africa reported that a statistically higher level of malondialdehyde (MDA) was observed in a group of patients with diabetes and complications compared to those without complications this finding compatible with study under hand (17). The high concentration of serum lipids in the type 2 diabetic subjects is mainly as a results of increased mobilization of free fatty acids from peripheral depots, due to loss of the inhibitory action of hormone sensitive lipase. Peroxidation of polyunsaturated fatty acids in blood produces malondialdehyde (MDA) that leads to oxidative damage. T2DP has reported an increased levels of lipid peroxidation (MDA) and reduced antioxidant status may play a major role in diabetic complications (25). Pavithra et al. reported higher levels of MDA in patients with DM compared with controls and in patients with DR versus those without DR, also suggesting that oxidative stress may play a significant role in the development of DR. As a result, levels of MDA are used as an index of LPO and in consequence of oxidative stress (2). Hyperglycaemia leads to overproduction of ROS through several pathways that may lead to destruction of various macromolecules in the body including lipids through the mechanism of oxidative stress (26).

**Financial disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Chemistry and all experiments were carried out in accordance with approved guidelines.

**References**


Evaluation of Marginal adaptation at Interfaces Using Composite Resin to Different Setting Amalgam Filling in Class II Cavity Preparation

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Abstract

Aim: The objective of the present study was to evaluate the microleakage of composite restorations alone or with amalgam base in proximal box of class II cavity preparation.

Method: Eighty proximal cavities (Mesio-occlusal and disto-occlusal) were prepared in 40 premolars with carbide bur and randomly divided into four groups. Group A restored with composite resin; the other three groups restored with combined amalgam-composite restoration as follows. Group B the composite inserted immediately after insertion of amalgam, group C the composite inserted after 5 minutes of amalgam insertion while in group D the composite inserted after two days of amalgam insertion. Marginal adaptation was evaluated at the following interfaces: tooth-amalgam(T-A), tooth-composite resin (T-C) and amalgam-composite (A-C). Microleakage was evaluated by means of methylene blue infiltration after 21 days water storage and thermocycling aging. Microleakage was assessed by measuring the depth of horizontal dye penetration. Image J analysis software was used to measure the penetration of dye in the tooth- filling interface and in the amalgam-composite interface. One-way ANOVA and post-hoc Tukey HSD tests (α =0.05) were used statistically for analyzing gather data.

Results: There were a significant statically showed by ANOVA test between teeth -fillings interface, according to Tukey’s test there were a significant difference between teeth-composite interface and teeth-amalgam interface (p<0.05), while between composite- amalgam interface, ANOVA test showed a significant difference between groups and Tukey’s test showed a significant difference between groups according to time of placement of composite on amalgam filling (p<0.05).

Conclusion: Using of amalgam at the cervical base of the box in Class II combined amalgam-composite restoration has better result than using of composite from marginal leakage view.

Keywords: microleakage, marginal adaptation, Class II cavity, amalgam-composite restoration.

Introduction

The restorations of amalgam have good mechanical properties and many advantages such as relatively easy of insertion, resistance to fracture are adequate reasonably, after a period of time it allows good marginal sealing in the mouth, relatively economical and its less sensitive technique than direct tooth colored composite restorative materials (¹). Historically, it was used for long time clinically with proven longevity (²). In spite of initial marginal adaptation of composite resin restoration is better than amalgam (³), amalgam restorations occasionally fail because of secondary caries (⁴) and this due to deposition of oxides by surface corrosion which leads to enhance marginal self-sealing over time (⁵). The chief complaint of amalgam restorations is the darkness of color of these restorations which presents an unpleasant aesthetically (⁶). On the other hand, tooth colored composite resin material represents an aesthetically pleasant materials for restorations which have many advantages such as
requiring less sacrificing of sound tooth structure and enhance fracture resistance of teeth (7). In spite of the failure rate of composites is similar to amalgam (8) but there are many shortcomings such as; it’s technique sensitive, it’s survival rate in larger cavities is less when compared to smaller cavities (9). It’s bonding in the gingival margins of proximal restorations are critical because of little enamel in this area or even diminished of it (10) and it’s failure may occurs due to marginal gaps that results due to polymerization shrinkage which leads to breakdown of adhesive bonding (11). This shortcoming of composite fillings in the gingival margin of proximal boxes are opposite to the good marginal seal of amalgam fillings in this area because of gradual oxide deposition (12). To benefits from the advantages of composite resin restorations and amalgam restorations and to minimize their shortcomings, a combined of composite and amalgam restorations can be an alternative for them separately (13). The advantage of this combination restorations; the amalgam ensures the cervical marginal auto-sealing with aging while composite strengthen residual dental structures in addition to it’s esthetically acceptance (14,15). Another advantage of this possible combination is the repair of fractured of old amalgam restoration without the need for removing the remaining intact amalgam (16).

Materials and Methods

Forty readily available intact human caries-free maxillary premolars were collected and stored in distilled water. All teeth were cleaned and polished using pumice and rubber cups. The teeth were mounted in the acrylic resin to within 2 mm apical to the cemento-enamel junction to facilitate the handling and control of the samples. on these teeth eighty standardized mesio-occlusal (MO) and disto-occlusal (DO) Class II box cavities were prepared with gingival margins located 1 mm above the CEJ. Each tooth received two proximal cavities using a high-speed handpiece with air/water spray. A new bur was used for each four teeth. The bucco-lingual width of the cavities was approximately one-third of the intercuspal distance, and the depth were 2.5 millimeters.

The samples were randomly divided into four groups (n=10 (20 proximal cavities) /group) to receive the following treatment:

Group A: Restored with Composite resin.

Group B: Restored with combined amalgam-composite (composite placed immediately after insertion of amalgam).

Group C: Restored with combined amalgam-composite (composite placed after 5 minutes of insertion of amalgam).

Group D: Restored with combined amalgam-composite (composite placed after 2 days of insertion of amalgam).

The Composite resin used in treatment was (SonicFill 2, Kerr A2), the amalgam was (F-400, SDI), the Bonding agent was (Scotchbond™ Universal Adhesive, 3M-ESPE), the light cure used for polymerization of composite was (SDI Radii-Plus), the amalgamator used for trituration of amalgam was (SDI Ultradent 2, Victoria, Australia) and the matrix bands used were (SuperMat Adapt SuperCap Matrix, Kerr) which were used for all teeth before starting treatment.

In group A: the cavities were etched using phosphoric acid 37% for 15 seconds then thoroughly rinsed with water and dried then bonding applied as recommended by manufacturer’s instruction then SonicFill 2 composite resin were applied in two increments to fill the hole cavity and each increment was light cured as manufacturer’s instruction recommendation.

In group B: after etching the cavities, the alloys were mixed according to manufacturer instructions using an amalgamator. A standardized technique was used to transfer the amalgam to cavities using amalgam carrier until reaching the height of pulpal floor. Bonding agent was applied and cured immediately after insertion of amalgam and then composite resin was applied to the remaining cavity in one increment and cured as recommended by manufacturer’s instruction.

In group C: after etching of cavities, the alloys were mixed, transferred to cavities until reaching the height of pulpal floor. After 5 minutes of amalgam insertion, bonding agent was applied and cured and then composite resin was applied to the remaining cavity in one increment and cured as recommended by manufacturer’s instruction.
In group D: after etching of cavities, the alloys were mixed, transferred to entire cavities with minimal carving and after setting of amalgam the samples were stored in distilled water. After two days of amalgam insertion, 1.5 mm of amalgam filling was removed then bonding agent was applied and cured and then composite resin was applied to the new remaining cavity in one increment and cured as recommended by manufacturer’s instruction.

All the samples were stored in distilled water after restorations for 21 days, then thermocycling were performed at 5 ± 1°C to 55 ± 1°C for 500 cycles, with 30 seconds dwell time. The teeth were coated with two layers of nail varnish except for the restoration and 1 mm of peripheral area, then stored in container containing 2% Methylene Blue for 24 hours at room temperature. After removal from the dye solution, teeth were rinsed under running tap water and left at room temperature for 2 hours for dryness and dye fixation then embedded in clear resin. The specimens were sectioned in mesio-distal direction at the middle of the restorations with special diamond sectioning bur (Renfert, Germany) mounted in dental engine (Marathon 3, Korea) with water cooling. Microleakage was evaluated using Image analysis (Image J) software by measuring the linear penetration of the dye in micrometer from the external surface of interfaces using a stereomicroscope with a reflected light under magnification of 45x. Marginal adaptation was evaluated at the following interfaces: tooth-composite resin interface (T-C), tooth-amalgam interface(T-A) and amalgam-composite resin interface(A-C). Descriptive statistics including means and standard deviations were calculated for the microleakage analysis. The obtained data were subjected to one-way analysis of variance (ANOVA) and Tukey HSD test to determine significant differences among the three interfaces. The level of significance was set at p =0.05. All statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

**Results**

A total of 80 sections were examined for evaluation of the dye penetration quantitatively at the tooth-filling interface and amalgam-composite interface with different periods of placement of composite. All the groups exhibited microleakage between the tooth-filling interface and amalgam-composite interface.

In the tooth-filling interface; The descriptive statistic (mean, standard deviation and standard error) are represented in (table 1). One-way ANOVA showed that the difference between the experimental groups was statistically significant (p<0.05) (Table 2). The post hoc Tukey multiple comparison tests showed the following results: tooth-composite interface exhibited statistically significant results compared with the three others amalgam-tooth interfaces(p<0.05), There was no statistically significant difference between groups of amalgam-tooth interfaces (p>0.05) (Table 3).

In the amalgam-composite interface; the descriptive statistic (mean, standard deviation and standard errors) are represented in (table 4). One-way ANOVA showed that the difference between the experimental groups was statistically significant (p<0.05) (Table 5). The post hoc Tukey multiple comparison tests showed the following results: the microleakage between fillings when composite inserted after two days of amalgam insertion exhibited statistically significant results compared with the others two groups (p<0.05), There was no statistically significant difference in microleakage between fillings whether the insertion of composite immediately or after 5 minutes of amalgam insertion (p>0.05) (Table 6).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Descriptives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filling, Tooth</td>
<td>N</td>
</tr>
<tr>
<td>composite immediately after A.F.</td>
<td>20</td>
</tr>
<tr>
<td>composite after 5 minutes of A.F.</td>
<td>20</td>
</tr>
<tr>
<td>composite after 2 days of A.F.</td>
<td>20</td>
</tr>
<tr>
<td>composite only</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
</tr>
</tbody>
</table>
Silver amalgam was widely used for restorations of posterior teeth due to their unique properties but with the improvement of tooth colored restorations, the drawback of amalgam fillings mostly due to their unpleasant color. In spite of composite bind micro-mechanically to enamel and dentin but unfortunately, its inherent polymerization shrinkage leads to leakage of oral fluids and toxins of bacteria leading to secondary caries especially in proximal cavities at cervical areas (17). One of the methods to overcome this shortcoming of composite is the combined amalgam composite restoration in which the amalgam inserted in the base especially in the cervical area under the composite (18), in order to combining the aesthetic properties of composite and required properties of amalgam cervically.

In the present study, the interfaces between tooth-composite, tooth-amalgam and composite-amalgam were evaluated using dye penetration test which considered one of the most commonly employed test used in microleakage studies. The linear penetrated dye was measured using Image J analysis software to have quantitative outcomes rather than subjective scoring.

Eighty interfaces between teeth and fillings were investigated to measure dye penetration, analyzed data showed that the composite have more leakage than amalgam and this may be due to the presence of little

Table 2

<table>
<thead>
<tr>
<th>Filling Tooth</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
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<tr>
<td>Between Groups</td>
<td>2235666</td>
<td>3</td>
<td>750855.012</td>
<td>28.631</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>1993111</td>
<td>76</td>
<td>26225.143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4245776</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3

Multiple Comparisons

<table>
<thead>
<tr>
<th>(i) Groups</th>
<th>(j) Groups</th>
<th>Mean Difference (i-j)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>composite immediately after A.F.</td>
<td>composite immediately after A.F.</td>
<td>-7.00</td>
<td>51.210</td>
<td>.999</td>
<td>-141.52</td>
</tr>
<tr>
<td>composite after 5 minutes of A.F. composite after 2 days of A.F.</td>
<td>composite only</td>
<td>-39.556</td>
<td>51.210</td>
<td>.867</td>
<td>-174.07</td>
</tr>
<tr>
<td>composite immediately after A.F.</td>
<td>composite after 2 days of A.F. composite only</td>
<td>-401.506*</td>
<td>51.210</td>
<td>.000</td>
<td>-536.02</td>
</tr>
<tr>
<td>composite after 5 minutes of A.F. composite after 2 days of A.F. composite only</td>
<td>-7.00</td>
<td>51.210</td>
<td>.999</td>
<td>-127.52</td>
<td>141.52</td>
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<tr>
<td>composite immediately after A.F.</td>
<td>composite after 5 minutes of A.F. composite only</td>
<td>-32.556</td>
<td>51.210</td>
<td>.920</td>
<td>-167.07</td>
</tr>
<tr>
<td>composite after 5 minutes of A.F. composite only</td>
<td>-394.506*</td>
<td>51.210</td>
<td>.000</td>
<td>-529.02</td>
<td>-259.98</td>
</tr>
<tr>
<td>composite after 2 days of A.F. composite immediately after A.F.</td>
<td>composite after 5 minutes of A.F. composite only</td>
<td>-39.556</td>
<td>51.210</td>
<td>.867</td>
<td>-94.97</td>
</tr>
<tr>
<td>composite immediately after A.F.</td>
<td>composite after 5 minutes of A.F. composite only</td>
<td>-32.556</td>
<td>51.210</td>
<td>.920</td>
<td>-167.07</td>
</tr>
<tr>
<td>composite after 5 minutes of A.F. composite only</td>
<td>-361.906*</td>
<td>51.210</td>
<td>.000</td>
<td>-496.47</td>
<td>-227.43</td>
</tr>
<tr>
<td>composite only composite immediately after A.F.</td>
<td>composite immediately after A.F.</td>
<td>401.506*</td>
<td>51.210</td>
<td>.000</td>
<td>266.98</td>
</tr>
<tr>
<td>composite after 5 minutes of A.F. composite only</td>
<td>394.506*</td>
<td>51.210</td>
<td>.000</td>
<td>259.98</td>
<td>529.02</td>
</tr>
<tr>
<td>composite after 2 days of A.F. composite only</td>
<td>361.906*</td>
<td>51.210</td>
<td>.000</td>
<td>227.43</td>
<td>496.47</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level.

Table 4

Descriptives

<table>
<thead>
<tr>
<th>Amalgam_Composite</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>composite immediately after A.F.</td>
<td>20</td>
<td>87.95</td>
<td>39.276</td>
<td>8.782</td>
<td>69.57</td>
<td>106.33</td>
<td>46</td>
<td>179</td>
<td></td>
</tr>
<tr>
<td>composite after 5 minutes of A.F. composite immediately after A.F.</td>
<td>20</td>
<td>85.60</td>
<td>30.684</td>
<td>6.661</td>
<td>71.24</td>
<td>99.96</td>
<td>32</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>composite after 2 days of A.F. composite immediately after A.F.</td>
<td>20</td>
<td>76.50</td>
<td>35.777</td>
<td>7.688</td>
<td>108.99</td>
<td>142.01</td>
<td>60</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>99.68</td>
<td>39.250</td>
<td>5.067</td>
<td>89.54</td>
<td>109.82</td>
<td>32</td>
<td>181</td>
<td></td>
</tr>
</tbody>
</table>
or even no enamel in the cervical area which is the main cause of decreasing bond strength in this area. In spite of the advancement in composite and bonding system which may be the cause of not extending the dye more than 1 mm in the interface between tooth and composite but still this may leads to caries which subsequently jeopardize the longevity of tooth integrity; in contrast, the gap sealing between tooth and amalgam restoration may be time dependent.

On the other hand, the extent of microleakage in the amalgam-composite interfaces in the present study showed that the leakage when insertion of composite immediately after insertion of amalgam was less than leakage when insertion of composite on set amalgam or even less than waiting five minutes, and this may be due to penetration of bonding agent into the freshly placed amalgam inside the irregularities and porosities of it’s surface and as a consequence decreasing of microleakage in the interface by creating bonding with composite resin filling and this in agreement with Mertz-Fairhurst & Newcomer 1988 (19) and Sharafeddin & Moradian 2008 (20).

The maximum amount of dye penetration in the amalgam-composite interface in the present study was 179 micron which may be considered as an acceptable value especially in a freshly placed amalgam because there is a possibility of sealing of this gap by oxide layer with time. The result of this study indicates that the repairing of existing amalgam filling restoration by composite resin materials would be more advantageous from the view of conservative dental treatment and from the view of esthetic consideration and this in agreement with Çehreli et al. (21).

Conclusions

Although all interfaces of restored specimens in the present study showed some degree of microleakage but the findings may be clinically acceptable especially in placement of composite on freshly placed amalgam in class II combined amalgam-composite restoration which also may indicate a reliable method for restoring defective amalgam restoration.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved and all experiments were carried out in accordance with approved guidelines.

References


Brain habits, Accuracy and Its Relationship to Self-Confidence for Handball Players

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Abstract

The current research aims to identify the struggle for accuracy and its relationship with self-confidence among players of the Baghdad and Middle Euphrates clubs with handball, to achieve this goal, two tools have been prepared, one of which is to measure the struggle for accuracy and the other to measure self-confidence after finding their psychometric characteristics represented in honesty and constancy, the two tools were applied on the research sample of (152) players from the clubs of Baghdad and the Middle Euphrates with youth handball and after analyzing the data using statistical means the research resulted in the research sample enjoying the struggle for accuracy and self-confidence in them, as well as the existence of a statistically significant correlation between the struggle for accuracy and self-confidence of the players of Baghdad and Euphrates clubs with handball.

Keywords: Brain habits, self-confidence, handball players

Introduction

Athletes generally try to achieve their own goals and they differ in their responses to different alerts and situations according to individual differences, the situation that includes a challenge to the player may be threatening to another player, and this is why he encourages 1 the first to deal with this situation and tries to solve the problem he is exposed to and sees in it an opportunity to enhance himself and his appreciation and increase his sense of self-confidence and confront the opponent with high spirit and ability as a result of the size of the stadiums, especially the handball court, due to the approach of the opponent and the continuous increase in pressure along the competition, the psychological 2 aspect plays an important, prominent and direct role, and the lack of interest in it leads to a lack of fulfillment of the duties, technical and planning skills, and adaptation to the opponent’s reactions, through which the behavior of the players on the field can be determined with high and accurate performance, and this requires a continuous physical, 3 skill and psychological struggle. One of the habits of the mind is (the struggle for accuracy). When a player matures in possessing this trait, he increases the care and focus of his work at the required level in the stadium this trait is related to what the player possesses in terms of cognitive, skill and psychological characteristics and characteristics, and it may have one of these traits, which is the subject of this research, which is self-confidence, and in that, given that a person’s confidence in his abilities, skills and attributes is a catalyst factor to improve his performance, whether that performance is technical, physical or mental 4. Hence, the problem of the current research is summarized in the answer to the following question: What is the strength and direction of the correlation between the struggle for accuracy and self-confidence of the players of the Baghdad clubs and the Middle Euphrates with hand reel?

Research objectives:

- The struggle for accuracy among the players of the Baghdad and Euphrates clubs with handball.
- Self-confidence of the players of the clubs of Baghdad and the Euphrates through handball.
Relationship the correlation between the struggle for accuracy and self-confidence of the players of the Baghdad clubs and the Middle Euphrates handball.

Research fields:

The human field: Includes players from Baghdad clubs middle Euphrates youth.

Time field: from 15/7/2020 to 22/10/2020.

Spatial field: Club halls in Baghdad and the middle Euphrates.

Terminology used:

- The struggle for accuracy:

Definition (Kallick & Costa, 2000): Individuals who value accuracy and perfection and take the time to check their products and to make sure that what they do is accurate and flawless in order to reach the highest possible standards and this makes them proud of their achievements that are characterized by perfection, craftsmanship and perfection(1).

Procedural definition: The total score that the respondent obtains when answering the paragraphs of the Struggle for Accuracy scale adopted in the current research.

- Self-confidence:

The researchers definition it: It is a personality trait of a strong and balanced individual and his ability to deal with different situations and the direction of others by knowing himself as a result of continuous evaluation and appreciation.

Procedural definition: The total degree obtained by the respondent when answering the paragraphs of the self-confidence scale adopted in the current research.

Research methodology and field procedures:

Research Methodology:

The researcher used the descriptive approach in the survey method and correlational studies as a better way to solve the research problem due to its relevance and the nature of the current study.

Community and sample research:

The research community included (250) young handball players from clubs in Baghdad and the Middle Euphrates. The research sample included (*). By applying the (Stephen) equation (*) to (152) youth players from Baghdad’s clubs with handball, it was withdrawn by a simple random method, as shown in table (1) below.

\[ n = \frac{N \times p (1 - p)}{\left[ N - 1 \times \left( \frac{d^2}{z^2} + \frac{2}{N} \right) \right] + p (1 - p)} \]

Table (1) shows the numbers of community members and the research sample and the percentages for each club:

<table>
<thead>
<tr>
<th>N</th>
<th>Clubs</th>
<th>Research community</th>
<th>Research sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Members</td>
<td>Percentage</td>
</tr>
<tr>
<td>1</td>
<td>Clubs of Baghdad</td>
<td>65</td>
<td>26%</td>
</tr>
<tr>
<td>2</td>
<td>Karbala</td>
<td>45</td>
<td>18%</td>
</tr>
<tr>
<td>3</td>
<td>Babylon</td>
<td>50</td>
<td>20%</td>
</tr>
<tr>
<td>4</td>
<td>Diwaniya</td>
<td>45</td>
<td>18%</td>
</tr>
<tr>
<td>5</td>
<td>Najaf</td>
<td>45</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>250</td>
<td>%100</td>
</tr>
</tbody>
</table>
Research tools:

In order to achieve the goals of the current induction, two tools were required, one to measure the struggle for accuracy and the other to measure self-confidence, through reviewing the literature and previous studies, a scale (Struggle for Accuracy) prepared by (Asaad Abdul Hassan: 2019), In building this scale, it was based on the theory (Costa and Calic: 2000) of the habits of the mind with its (71) paragraphs and its (five) dimensions, and the measure of self-confidence prepared by (Marwa Abdul-Jabbar: 2014) with its (60) paragraphs and its (three) dimensions.

For the purpose of applying the two scales, it was necessary to verify their psychometric properties represented in validity and consistency, as follows:

Validity: Means that the test measures what it was set for, meaning that the honest test measures the job that it claims to measure and does not measure anything else in place of it or in addition to it (3). Therefore, the two scales were presented to a group of specialized arbitrators, the results have resulted through (deleting, amending and merging) the paragraphs of the two main scales, as shown in table (2) below.

Table (2) shows the opinions of specialists in the paragraphs of the scale of struggle for accuracy, the Scale of self-confidence, and the Chi-square for agree and disagree.

<table>
<thead>
<tr>
<th>Struggle for accuracy scale</th>
<th>Self-confidence scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paragraphs</td>
<td>Agree</td>
</tr>
<tr>
<td>1.2.10.13.15.16. 18.21 22.24.30.33.50.55.60.65.</td>
<td>10</td>
</tr>
<tr>
<td>34.35.36.37.38.39.40.41.68</td>
<td>9</td>
</tr>
<tr>
<td>3.4.5.6.7.8.9.11.12.14.17.27.28 48.51.52.53.66.67.69</td>
<td>8</td>
</tr>
<tr>
<td>19.20.23.25.26.29.31.45.46.47 54.56.57.58.59.70</td>
<td>6</td>
</tr>
<tr>
<td>32.36.42.43.44.61.62.63.64.71</td>
<td>5</td>
</tr>
</tbody>
</table>

Chi-tabular value = 3.84 , degree of freedom = 1, significance level = 0.05

It is noted from table (2) above that the number of remaining paragraphs of the scale of (Struggle for accuracy) reached (25), because the value of the Chi calculated ranged between (10 - 6.4) which is greater than the tabular value (3.84) at the level of significance (0.05), and the degree of freedom (1) while (46) paragraphs were deleted because the value of chi calculated for it ranged between (0 _ 3.6), which is less than the tabular value of chi (3.84) at a level of significance (0.05) and a degree of freedom (1). As for the measure of self-confidence, (20) items were kept because the value of
Kai which was calculated for it ranged between (6.4-10), which is higher than the value of Kai of (3.84), While (40) paragraphs were deleted, the value of chi ranged between (0 _3.6), which is less than the tabular value of (Ki) of (3.84) at the level of significance (0.05) and the degree of freedom (1).

Reliability: A static test is a test that has a high degree of accuracy, proficiency, consistency, and objectivity in relation to its measurement (4), to achieve this, the two measures were applied to a sample of the youth group of (25) players, and after a two-week period, the two scales were re-applied to the same sample. according to the (Pearson) correlation coefficient, its value appeared (0.86), which is a good indication of the reliability of the Struggle for accuracy scale, and (0.80) for the self-confidence scale.

Statistical means: The statistical data were processed using the following laws:

1. The arithmetic mean
2. Standard deviation
3. T test for a sample and the community
4. Pearson Correlation Coefficient
5. The chi-square test for good matching

The final application: After the psychometric characteristics were found, represented by validity and reliability in the scale of the struggle for accuracy and self-confidence, they were applied to the research sample of (152) players from the clubs of Baghdad and the Middle Euphrates with handball.

Presentation, analysis and discussion of results:

This chapter includes a presentation, explanation and discussion of the findings of the researchers after analyzing the answers and performance of the sample members and treating them with appropriate statistical means as follows:

In order to achieve the first objective of the research, which is to identify the level of struggle for accuracy of the clubs of Baghdad and the Middle Euphrates with handball, the answers of the individuals of the research sample (152) were analyzed, and it appeared that the arithmetic mean reached (93) with a standard deviation (8.6), which is higher than the hypothesis mean of (62.5) to verify the differences between the arithmetic mean achieved and the hypothesis, a t-test was used for one sample, and the results appeared in table (3) below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>mean</th>
<th>Std. deviation</th>
<th>hypothesis mean</th>
<th>T value</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>93</td>
<td>8.6</td>
<td>62.5</td>
<td>43.36</td>
<td>Sig</td>
</tr>
</tbody>
</table>

It appears from table (3) above that the calculated value of (t) of (43.36) is higher than the tabular value of (t) of (1.96) at the level of significance (0.05) and the degree of freedom (151), this means that the research sample represented by (youth handball players for Baghdad and Euphrates clubs) have a high level of struggle for accuracy, this may be attributed to the nature of the game practiced by these players and the high ability to focus on performance to achieve achievement, and this high ability stems from the fact that the members of the sample are in most of them students of the Faculties of Physical Education and Sports Sciences, which was positively reflected on their performance considering that the vocabulary of the school curriculum and the information it contains were reflected in their motivation and desire for the better.

And to achieve the second goal of the research, which is to identify the level of self-confidence of (players of Baghdad and Euphrates clubs with handball), after analyzing their answers, it was found that the arithmetic mean (83) with a standard deviation (6.02), which is higher than the hypothesis mean (60), and
for the purpose of identifying the statistical significance of the apparent differences between the arithmetic and hypothetical mean, test (T) was used for one sample, the results appeared as shown in table (4) below:

**Table (4) Shows the arithmetic and hypothetical average and the calculated T-value for the sample of Baghdad and Euphrates clubs for the measure of accuracy and self-confidence**

<table>
<thead>
<tr>
<th>Sample</th>
<th>mean</th>
<th>Std. deviation</th>
<th>hypothesis mean</th>
<th>T value</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>83</td>
<td>6.2</td>
<td>60</td>
<td>11.5</td>
<td>Sig</td>
</tr>
</tbody>
</table>

It emerged from table (4) above that the calculated value of (t) amounted to (11.5), which is higher than the tabular value of (t) of (1.96) at the level of significance (0.05) and the degree of freedom (151), this means that the research sample represented by (youth handball players for Baghdad and Euphrates clubs) have self-confidence, as previously mentioned, it comes from two sources, one of which is represented by the psychological and cognitive culture that the players possess, since most of them are students of the Faculties of Physical Education, so they kept their practice of this game and their mastery of its skills, which was positively reflected in raising their self-confidence.

To achieve the third goal of identifying the correlation between (the struggle for accuracy and its relationship to self-confidence), a Pearson correlation coefficient was used that reached (0.72), for the purpose of identifying the statistical significance of the correlation coefficient, the (t-t) test was used, as shown in table (5) below.

**Table (5) Shows the correlation coefficient of the research sample.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>correlation coefficient</th>
<th>T .R</th>
<th>T value</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>0.72</td>
<td>12.87</td>
<td>1.96</td>
<td>Sig</td>
</tr>
</tbody>
</table>

It appears from table (5) above that the calculated value (T .R) of (12.87) is higher than the tabular value of (T) of (1.96) at a level of significance (0.05) and a degree of freedom (150). The more keen and eager to achieve accuracy in performance and this is what appeared through this result.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved and all experiments were carried out in accordance with approved guidelines.

**References**

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Chronic Myeloid Leukemia and Associated Oral Manifestations

Suha Abdulhussein Hindy1, Ali M. H. Alyassiri2, Ali Q.L. Lilo3

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Abstract

Background: Leukemia defined as a hematological disorder associated with proliferating white blood cell-forming tissues as resultant increasing of circulating immature white blood cells or abnormal cells.

Aims of the Study: To investigate clinically the oral manifestations or discomfort oral features in patients with chronic myeloid leukemia.

Subjects, Materials and Methods: Thirty- three patients participated (17 males and 16 females) in the current study with chronic myeloid leukemia already.

Results: Total numbers of cases were (33). (17 males and 16 females), the percentages of these results showed gingival bleeding (87%), fungal infection (84%), oral petechiae (72%), gingival overgrow (63%), and then finally painless oral ulceration (60%), The early diagnosis of this disease knowing that the first clinical symptoms of leukemia happen in the oral cavity with normal or show challenging changes in initial laboratory tests.

Conclusions: Because of their clinical importance for early diagnosis, all such lesions or pathological status deserves the full attention of the doctors (like oral physician and general physician).

Keywords: Leukemia, oral signs, complications.

Introduction

Lymphoma, leukemia and finally plasma cell tumors; are the most common groups of hematologic malignancies. Leukemia is defined hematological disorder associated with proliferating white blood cell-forming tissues as resultant increasing of circulating immature white blood cells or abnormal cells. Leukemic disease characterized by a disordered proliferation and differentiation of neoplastic cells. Leukemia, arises from the clonal proliferation of abnormal hematopoietic cells with impairment in regulation, differentiation, and finally in programmed cell death “apoptosis”. The multiplication of leukemic cell at the expense of normal hematopoietic cell lines results failure in marrow area, depressing in the count of blood cell “cytopenia” and bleeding then finally death due to infection, or both outcomes 1,2. The exact cause of leukemic disorder is unknown. Certain chemicals (benzene), large doses of ionizing radiation, then finally infection with specific viruses (e.g., Epstein-Barr virus “EPV”, human lymphotropic virus) these factors associated with increasing causes of leukemia. Exposure to electromagnetic fields and cigarette smoking also have been proposed to be causative conditions 3. Depending on clinical behavior; leukemia classified into acute or chronic, also can be characterized based on hematopoietic cell line affected myeloid or lymphoid. Clinically four diagnostic categories of this disorder can be clarify as fallow 4,5:

1. “acute myelogenous leukemia AML”,
2. “acute lymphocytic leukemia ALL”.
3. “chronic myelogenous leukemia CML”. and
finally

4. **“chronic lymphocytic leukemia CLL.”**

Seventy percent of leukemia patients revealed in the line of their disease oral symptoms or signs. The most obvious oral features or manifestations in patients with leukemia are petechiae due to thrombocytopenia, ecchymosis, and gingival hemorrhage or finally gingival bleeding.

Ecchymosis is involved in the differential diagnosis, a coagulation disorder or hemorrhagic diathesis. Certainly, patients taking anticoagulant drugs may have associated with oral ecchymosis, especially on the buccal mucosa area or tongue sided; either of which can be traumatized while chewing process. Appearing of ecchymosis in the oral mucosa may also related with liver cirrhosis patients finally end-stage renal disease exposing dialysis technique or also called patients with chronic renal failure (CRF).

Gingival overgrowth or enlargement is usually associated with local inflammatory conditions such as food impaction, poor oral hygiene, or mouth breathing. Systematic events like drug therapy, hormonal changes, or infiltration by tumor may also cause or predispose to the severity of gingival enlargement. The gingival overgrowth or hyperplasia can be noted in von Recklinghausen’s neurofibromatosis “neurofibromatosis 1”, sarcoidosis, Wegener’s granulomatosis, primary amyloidosis, Crohn’s disease, acromegaly, Kaposi’s sarcoma, and then finally lymphoma and patients with leukemia.

The development or clinical appearance of gingival infiltration is unpredictable in any individual patient. As general talking, gingival hyperplasia or overgrowth resolves completely or partially with effective type of treatment in patients with leukemia; as chemotherapeutic type.

Oral signs and symptoms or clinical features, in patients with leukemia may include; painless mucosal ulceration, also the oral infections a typically present, as dental abscesses may clinically appear as necrotizing area in oral soft tissue without swelling, and recrudescent herpes simplex virus, may present with wide extending lesions affecting both the keratinized mucosa and non-keratinized mucosal area, also oral soft tissue colonize with oral Candida albicans.

**Aims of the study**

To investigate clinically the oral manifestations or discomfort oral features in patients with chronic myeloid leukemia.

**Subjects, Materials and Methods**

Thirty three subjects participated (17 males and 16 female patients) in the current study with chronic myeloid leukemia already diagnosed by hematologist specialists in Merjan medical city / Babylon – Iraq, this research was done between December 2016 - April 2017. These subjects without signs and symptoms for any other medical history. The intra-oral examination of patients were done during the first two to four weeks of diagnosis.

Intra-oral photographic pictures were taken for each patient; dental mirror, tweezer and pieces of gauze were used during examination and diagnosis of these oral lesions.

**Results**

The results of the current study as shown in the table (1).

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Oral petechiae, hemorrhage</th>
<th>Gingival bleeding</th>
<th>Gingival overgrowth</th>
<th>Painless Oral ulceration</th>
<th>Candidal infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 (100%)</td>
<td>24 (72%)</td>
<td>29 (87%)</td>
<td>21 (63%)</td>
<td>20 (60%)</td>
<td>28 (84%)</td>
</tr>
</tbody>
</table>

To clarify purpose, these results showed in the following, figure (1).
That means. Higher percentage of patients (87%) present with gingival bleeding (fig. 2), followed by candidal infection (84%), (fig. 3), then oral petechiae (72%), then gingival overgrowth (63%), (fig. 4, a &b) and the least one is painless oral ulceration (60%), (fig. 5). That means the most common and first sign of chronic myeloid leukemia is gingival bleeding which considered as triggered sign in this disease, due infiltration of tumor cells in the gingival tissue.

Figure (1): Oral features in Patients with chronic myeloid leukemia.

Figure 2: Gingival bleeding  Figure 3: Fungal infection

Figure 4 (a): Gingival swelling  Figure 4 (b): gingival overgrowth
Discussion

As general talking, the appearance of oral features or manifestations in patients with leukemia according to general conditions of the patients. Before treatments begin for these patients, large numbers of leukemic cells infiltrates extending of wide area of oral tissues. 

Silva et al., 2012 revealed that the typical oral features of patients with leukemia in acute status or stage involving: oral ulceration, gingival swelling, petechiae, mucosal pallor, spontaneous gingival bleeding, then finally herpetic infections and candidiasis. Advanced cases of leukemia may include cervical lymphadenopathy, malaise, also include laryngeal pain and fever.

Clinical appearance of oral infection like fungal, herpetic and bacterial due to immunocompromised in health status in acute stage of disease in addition to the appearance of these opportunistic infections because of neutropenia during chemotherapy or radiotherapy.

Leukemia cells infiltrate tissues like oral soft tissues and membrane in addition to multiple functional organs (as lung, bones, testis, lymph nodes and so on… etc) that will become centaury sites or also called sites of infections (reservoirs).

Albuquerque et al., 2005 stated that the leukemia may resulted in destructive radiolucencies with losing of lamina dura around the teeth and erosion of the dental or crestal alveolar bone.

Finally, patients with leukemia may clinically associated with petechial patches, points, ecchymosis and bleeding tendencies due to blood vessels; larges and smalls infiltrates by leukemic cells also this disease including functionless and immature of blood cells therefore can be concluded the oral health considering as mirror images of general health.

The present study confirmed the same oral manifestations that manifested in previous studies, and the main manifestation is gingival bleeding.

Conclusions

The dentist, and mainly the periodontics and oral medicine specialists (oral physician), oral pathologist, plays an important fundamental role in the early diagnosis of this disease knowing that the first clinical symptoms of leukemia happen in the oral cavity, which mainly starts as spontaneous gingival bleeding with normal or show challenging changes in initial laboratory tests.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Oral Surgery and Oral Diagnosis and all experiments were carried out in accordance with approved guidelines.

References


Legalizing speed Endurance Training According to Some Physiological Indicators for 800-Meter Runners for Youth

Mohammed Amanah Kaittan1, Emad Amanah Kaittan2, Ahmed Abdulrazzaq Fadhil3

Abstract

The importance of research lies in the preparation and codification of speed endurance exercises according to some physiological indicators for 800-meter runners for youth and to identify the effect of codifying speed endurance training according to some physiological indicators of 800 meters for youth, as the researcher adopted the experimental approach in designing the two equivalent groups (control and experimental) to suit the nature of the research, where the research sample was determined by deliberate selection of young runners in athletics at the age of (18-19 years) according to the classification of the international athletics federation, as the research sample included (10) runners representing Baghdad clubs who were distributed randomly (by lot) into two groups control and experimental group each consisting of (5) runners, physiological tests were performed, including the maximum consumption of oxygen and lactic acid, and the physical tests included (1000) meter endurance test and (800) meter achievement test on the research sample, and the researcher came to the following conclusions that the speed endurance training gave progress in the level of the (800) meter running in this test, and they need to be stable and avoid fluctuation in the variation in the values of this test, the results showed that the experimental group members outperformed the control in the physiological indicators (lactic acid concentration, the maximum oxygen consumption (VO_{2max}) in the post-tests. The researcher recommended the necessity of conducting physiological tests to legalize training loads because of their effective role in the training process, focusing on developing and rationing the speed bearing capacity because of its direct impact on the development of achievement in the activities of middle-distance athletics.

Keywords: Legalizing speed, training, physiological indicators

Introduction

The development of the level of sports performance in order to achieve high levels of achievement in different sports depends on the diversity in the use of training methods that have a great impact in improving the sports figures and their different effects stimulate those interested in the field of sports training to the continuous diversity in the use of the most effective training methods, and a contribution to developing achievement. Therefore, the 800m game is one of the fast medium runs that is performed with sub-extreme intensity and due to this feature there is a specificity when training it in terms of the energy system prevailing in it and the method and training method that affects the physical abilities used and the physiological variables that characterize this game, and as a result of this peculiarity, the chosen physical capabilities and physiological indicators should be in this direction, because this event is viewed as a fast running race, and this gives an idea of the general trend in thinking about developing achievement in this competition it should focus on special physical abilities and some physiological variables affecting this competition and the common characteristics between it and the fast running and developing the \(^{3-5}\) time of this activity that needs compatibility and harmony, and because the runner possesses the capabilities that are in harmony with the requirements imposed by the performance stages of this event from the special physical abilities that have a positive effect in maintaining the speed throughout the race distance and the intensity required by the activity.
by taking advantage of improving neuromuscular work and adapting body systems in order to reach high achievement, hence the importance of research in codifying speed endurance training exercises according to some physiological indicators for 800-meter runners.

Research problem:

Run 800 Olympic competitions that require the athlete who specializes in this event to have special physical and physiological abilities in addition to patience, challenge, and willpower all combined in order to achieve achievement, the researcher noticed, through looking at the results and participation of the Iraqi runners, the achievement of the (800)m run, that the level of achievement in this event does not develop among Iraqi players compared to the international record that was recorded in the Brazilian Rio Janeiro olympics, as the scientific research and experiments that have been reached in this activity, which is not bearing large energy waste, the researcher was called upon to address this problem by codifying speed endurance exercises for the effectiveness of (800)m jogging according to physiological indicators, including the accumulation of lactic acid and the maximum oxygen consumption.

Research objectives:

1. Preparing and codifying speed endurance training according to some physiological indicators for 800-meter runners for youth.

2. Identify the effect of codifying speed endurance training exercises according to some physiological indicators of (800)m for youth

Research hypothec:

There are statistically significant differences between the pretest and the post test in (physical and physiological tests for 800-meter young runners and in favor of the post-test.

Research fields:

The human field: Baghdad youth runners clubs.


Spatial field: College of Physical Education and Sports Sciences Stadium for Athletics / University of Baghdad.

Methodology

The researcher chose the experimental approach in designing the two equivalent groups (control and experimental) for its suitability with the nature of the research.

Community and sample research:

The research sample was determined by intentional selection of young runners in athletics at the age of (18-19 years) according to the classification of the International Athletics Federation, as the research sample included (10) runners representing Baghdad clubs, who were distributed randomly (by lot) into two control groups. Each experimental group consisted of (5) runners, as the control group implemented the training curriculum followed by the trainer, either the experimental group adopted the same approach except for the speed-endurance exercises prepared by the researcher and it is implemented.

Devices, tools and methods used in the research:

Methods of data collection:

- Arab and foreign sources.
- The International Information Network The Internet.
- Personal interviews for experts.
- Exploratory experience.
- Tests and measurements.
- Results registration form.
- Data dump form.
- Auxiliary work team
- Statistical methods.

Tools and devices used:

To obtain data and reveal facts, the researcher will use the following tools:

- Lactic acid device measurement in the blood (Lactate Pro) number 2.
- Maximum oxygen consumption measuring device (VO2Max).

Field research procedures:

Determine the physical and physiological tests used in the research:

Test ran 1,000 meters:

**Objective**: To measure the speed endurance for running a distance of (800) meters.

**Tools used**: track and field stadium, (12) stopwatches, and registration forms.

**Scientific conditions**: (Shaker Muhammad Al-Sheikly) prepared by the British Olympic coaches (Watts and Wilson) as a speed endurance test for running 800 meters (1), experts and trainers agreed that it is suitable for measuring the endurance of speed for running a distance of (800) meters.

**Performance description**: All five players were selected together to ensure the element of competition, as each player stood in his field, then the test began by giving players a directive to go behind the starting line to take the starting position from standing, upon hearing the start signal, the players set out to run and cover two and a half laps (1000 meters), and upon reaching the finish line the timing clocks were stopped, and the time spent by each player was read, and recorded in the scoring form.

Achievement test ran 800 meters:

**The purpose of the test**: to measure the effectiveness of 800 meters.

**Tools used**: track and field stadium, stopwatches that can measure more than one time during the test, assistants, registration form.

**Performance description**: The test was conducted in accordance with the conditions and regulations of the International Federation of Athletics, as were all tested together for the purpose of runners compete, and every runner in the field of dedicated running, after that, the test begins by instructing runners to go behind the starting line to take the starting position from standing, when the start signal is heard, the runners start running two laps on the track for a distance of 800 meters.

Register: The registrar records the completion time in the form prepared for this purpose in the minute and the second to the nearest fraction of a second.

**Blood lactic acid concentration test before and after physical effort** (2):

**Test name**: Measurement of lactic acid concentration in the blood before and after physical effort.

**Objective**: Find out the percentage of lactic acid concentration in the blood.

**Tools used**: A lactate pro2 LT-1710 device, needle drill, test strip, graduated tape, medical cotton, sterile materials and registration form.

**How to use it**: Blood samples will be taken every two weeks during the special endurance period before and after the effort.

Register: The reading shown by the device for each player is recorded in the registration form.

**Maximum oxygen consumption (VO2max) test** (3):

**Test objective**: Measurement of maximum oxygen consumption (VO2max).

**Device and tool**: Maximum oxygen consumption measuring device (VO2max).

**Procedures and performance specifications**: Before starting the test, the test performer cleans the VO2max respirator with an antiseptic solution, ties the Fitmate Pro system parts together, attaches the pulse belt to the tester’s chest, and attaches the Bluetooth pulse receiver to the Fitmate pro, after entering the player information into the device, which includes name, date of birth, gender, height, weight, and choosing the type of test to be performed (VO2mx), and then fixing the respirator tightly with its belts and making sure that breathing air does not leak from the mask, then the tester climbs onto the treadmills and runs gradually with increasing speed, as the tester begins to control the increase in running speed on the device with the speed gradient from the special button for that in the treadmills starting from (4.5) to (13-) 14) km / hour, the Fitmate Pro device contains a small screen with a graph box showing the pulse and maximum oxygen consumption (VO2max)
with the ratios for each of them, which is monitored by the rectifier.

**Register:** the device gives a comprehensive reading tape for measurements of maximum oxygen consumption (VO₂max), the image of which is shown.

**Measuring unit:** Milliliter / kg / minute.

**Exploratory experience:**

The exploratory experiment was conducted at nine o’clock in the morning on 1/9/2020 by three players from the research sample, in order for the players to get used to the testing procedures to understand them and not to be afraid of the procedures.

**Pre-tests:**

After completing the exploratory experiment and avoiding the difficulties and obstacles that appeared in it, the researcher conducted the pre-tests for the functional variables of the individuals of the research sample consisting of distance runners (800 meters).

The main experience:

The researcher prepared a training curriculum with endurance of speed for the effectiveness of (800 meters) based on his experience and field training experience and drawing on the opinions of specialists in the field of sports training science and scientific and Arab training and physiological sources. (Muhammad Reda Al-Madaghmi) confirms that most of the changes resulting from training occur during the first period of the curriculum Training within (8-12 weeks) (⁴). The duration of the implementation of the training curriculum lasted (8) weeks, at the rate of (2) two training units per week, and the number of training units reached (16) units that were applied in the period from 4/9/2020 to 3/11/2020.

**Post-test:**

The assistant work team, under the supervision of the researcher, conducted the post tests of the individuals, the research sample, and under the same circumstances, to give equal opportunity to the individuals of the research sample in recording the results.

The statistical means used:

- SPSS.
- (V20) (Virgin).
- Mean.
- Standard deviation.
- Coefficient of torsion.
- T-test for correlated samples.
- T-test for non-correlated samples.

Presentation, analysis and discussion of results:

**Table (1) Shows the results of the arithmetic mean and the standard deviations of the two research groups (experimental and control) in the pre and post- tests in the speed endurance test (1000 m) and the achievement test (800 m).**

<table>
<thead>
<tr>
<th>Physical abilities</th>
<th>Measuring unit</th>
<th>Group</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Std. deviation</td>
</tr>
<tr>
<td>Speed endurance (1000) meters ran.</td>
<td>Min /second</td>
<td>Experimental</td>
<td>160.625</td>
<td>2.96491</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>163.755</td>
<td>3.51759</td>
</tr>
<tr>
<td>Test of (800) meters run.</td>
<td>second</td>
<td>Experimental</td>
<td>125.034</td>
<td>1.749</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>126.249</td>
<td>1.214</td>
</tr>
</tbody>
</table>

N = 5 for each group
Table (1) shows that the arithmetic mean of the experimental group in the speed endurance test of running speed (1000) meters preceding was (160.625) and the standard deviation (2.9649), in the post test, the mean became (153.26) and the standard deviation (1.15877), while the mean of the control experimental group was (163.755) and the standard deviation (3.51759), and in the post test the mean became (160.989) and the standard deviation (3.04025). As for the achievement test, it shows that the mean of the experimental group in the pre-test was (125.034) and the standard deviation (1.749). In the post-test, the mean became (118.153) and the standard deviation (2.686), while the mean of the control group in the pretest was (126.249) and the standard deviation (1.214), and in the post test the mean became (123.383) and the standard deviation (2.359).

In order to identify the difference between the pre and post-tests in the results of the speed endurance test, the achievement test, the researcher used a (T-test) test for correlated samples for each of the two research groups, as shown in table (2):

Table (2) shows the mean of the differences, the deviations of the differences, the value of the test (T) and the significant significance of the two research groups (experimental and control) in the pre and post-tests in the speed endurance test (1000 m) and the achievement test (800 m).

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>Means difference</th>
<th>Std. difference</th>
<th>(T) Calculated</th>
<th>Sig level</th>
<th>Sig Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed endurance (1000)</td>
<td>Experimental</td>
<td>7.354</td>
<td>2.19259</td>
<td>7.508</td>
<td>0.004</td>
<td>Sig</td>
</tr>
<tr>
<td>meters ran.</td>
<td>Control</td>
<td>2.776</td>
<td>0.8685</td>
<td>7.139</td>
<td>0.004</td>
<td>Sig</td>
</tr>
<tr>
<td>Test of (800) meters run.</td>
<td>Experimental</td>
<td>6.880</td>
<td>3.923</td>
<td>3.922</td>
<td>0.015</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.868</td>
<td>1.975</td>
<td>3.249</td>
<td>0.035</td>
<td>Sig</td>
</tr>
</tbody>
</table>

Degree of freedom \((n-1) = 4\) and the level of significance \((0.05)\).

Table (2) shows that in a test with a running speed of (1000) meters, the mean difference in the mean between the pre and post-tests of the experimental group was (7.354) and the deviation of the differences was (2.19259) and the calculated value of the test (T-test) for the correlated samples was (7.508) at the level of Significance (0.05) and degree of freedom (4), and the value of (Sig) was (0.004), which is less than (0.05), which means the significance of the differences between the pre and post-tests in favor of the post-test. As for the achievement test, it shows that the mean difference in the mean between the pre and post-tests of the experimental group reached (6.880) and the deviation of the differences reached (3.923) and the calculated value of the (T-test) reached (3.922) and the degree of freedom (4), and the value of (Sig) (0.015) is less than (0.05), which means the significance of the differences between the pre and post tests and in favor of the post test, as for the mean difference in the mean between the pre and post-tests of the control group (2.776) and the deviation of the differences (0.8685), the calculated value of the (T-test) for correlated samples was (7.139) at a significance level (0.05) and a degree of freedom (4), and the value of (Sig) (0.004) which is less than (0.05), which means the significance of the differences between the pre and post-tests, and in favor of the post test. As for the achievement test, it shows that the mean difference in the mean between the pre and post-tests of the experimental group reached (6.880) and the deviation of the differences reached (3.923) and the calculated value of the (T-test) reached (3.922) and the degree of freedom (4), and the value of (Sig) (0.015) is less than (0.05), which means the significance of the differences between the pre and post tests and in favor of the post test, as for the mean difference in the mean between the pre and post-tests of the control group, it reached (2.868) and the deviation of the differences (1.975). The calculated value of the (T-test) was (3.249) at a significance level (0.05) and a
Degree of freedom (4), and the value of (Sig) (0.035) and it is less than (0.05), which means the significance of the differences between the pre and post tests and in favor of the post test.

Table (3) shows the mean, standard deviations, the value of (T) test and the level of significance between the post- tests between the experimental and control groups in the speed endurance test (1000 m) and the achievement test (800 m).

<table>
<thead>
<tr>
<th>Physical abilities</th>
<th>Test</th>
<th>Measuring unit</th>
<th>Group</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>(T) Calculated</th>
<th>Sig level</th>
<th>Sig Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed endurance</td>
<td>(1000) meters ran.</td>
<td>Min /second</td>
<td>Experimental</td>
<td>153.26</td>
<td>1.15888</td>
<td>5.310</td>
<td>0.001</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>160.986</td>
<td>3.04029</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test of (800) meters run.</td>
<td>second</td>
<td></td>
<td>Experimental</td>
<td>118.155</td>
<td>2.686</td>
<td>3.274</td>
<td>0.014</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>123.384</td>
<td>2.359</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Degree of freedom (n -2) $5 + 5 - 2 = 8$ and the level of significance (0.05)

Table (3) shows that the mean of the experimental group in the endurance test of running velocity was (1000) meters (153.26) and the standard deviation (1.15888). As for the control group, its mean was (160.986) and the standard deviation (3.04029). The calculated T-test value was (5.310) at the significance level (0.05) and the degree of freedom (8). (Sig) (0.001) which is smaller than (0.05). This means that there is a statistically significant difference with regard to the two research groups and in favor of the experimental group. As for the achievement test, it shows that the mean of the experimental group in the post test was (118.155) and the standard deviation (2.686), while the control group had its mean (123.384) and the standard deviation (2.359), after calculating the value of (T) calculated using the law of (T)-test for non-correlated samples that were (3.274) at a level of significance (0.05) and a degree of freedom (8), the value of (Sig) (0.014) is less than (0.05), which means that there is a statistically significant difference, while the two research groups in the achievement test ran (800) meters in favor of the experimental group.

**Discuss the Results**

Discussing the results of the difference between the pre and post tests for the two research groups and the (t) values for the post tests between the experimental and control groups in the speed endurance test (1000 m) and the achievement test (800 m).

From reviewing the two tables of statistical significance (2 and 3) for testing physical abilities, he ran (1000) meters between the pre and post-tests, the two research groups and the post-tests between them showed improvement in each of them in the tests and the superiority of the experimental group over the control group, and the researcher attributes this result to the proposed exercises that were done they applied them to an experimental group of young runners, which led to the occurrence of adaptations that had an effective impact on the development and development of speed endurance, and this is certainly due to the privacy of the proposed approach to developing special endurance, which is an important principle of sports training principles that must be taken into account, especially for running coaches (800
meters), Mufti Ibrahim Hammad believes that “one of the conditions for enduring special speed training as one of the physical abilities of anaerobic prolongation requires the use of exercises characterized by high intensity using the method of high-intensity interval training for specific periods of time interspersed with positive (incomplete) rest between repetitions, provided that these are The exercises are characterized by a gradual increase in velocity at a time when the distance also decreases gradually, which will improve the ability of velocity stretching. “(5) , Shakir Al-Dara’a states, “The results are better the more the intensity is close to the maximum intensity” (6). The researcher attributes this development in the dimensional tests and for the benefit of the experimental group in a test running (1000 meters) more than the racing distance to the effectiveness of special endurance exercises that contributed to the development of this test time, as the distance of this test is greater than the completion distance (800 meters), the test distance (1000 meters) needs both aerobic and anaerobic energy sources, therefore, the special endurance exercises used by the researcher tended towards the development of anaerobic capabilities and as a result of the repetitions and intensity specified for each distance, which led to the body carrying physical burdens as a result of its fatigue in order to create special adaptations for the functional organs that were reflected in the development of the performance level of this group in performing the maximum possible degree from the intensity of jogging and for the longest possible period of time, (Ibrahim Al-Basri) confirms, “Experiences have proven that endurance and stress in the body during exercise, especially special endurance exercises, lead to gradual normalization of the body on effort and thus affect the ability of the heart and circulation system (7), and the researcher believes that the exercises that have been implemented have contributed to creating functional changes and upgrading the level of group members, which enabled them to travel the test distance in the least possible time. Although the control group had obtained a percentage of development at the time of this test, the difference was in favor of the experimental group in the significance of the differences, as the regularity of the experimental group sample in performing the exercises and the commitment to the specified rest times without interruption and the seriousness that they enjoyed in performing the exercises of different distances, intensity and time contributed to raising the efficiency of the functional devices, modifies the vital functions of body systems and this effect appears in their responses to loads of different intensity (8).

As for the achievement of running (800 meters), the researcher attributes that this development that appeared on the members of the experimental group is due to the effectiveness of the proposed training approach adopted by the researcher, where he focused mainly on developing special endurance (endurance of speed) by the method of low and high interval training. The intensity is from the real competition time, which reflects the mutual relationship between endurance and speed in the term endurance speed as complex abilities that contribute to determining achievement during the race, (Raisan Khuraibet 1998) asserts that “structured and programmed training and the use of types of standardized stresses in training and the use of optimal rest types between repetitions lead to an improvement in the level of achievement (9), and this is what distinguished the experimental group from the control, which led to the development of the level of performance the experimental group, which was reflected in the performance of the maximum possible degree of racing speed and for the longest possible period of time, as the use of balanced and comprehensive training leads to avoiding a drop in running speed because special training works to improve endurance (10) , it must be noted that the proposed training curriculum that the experimental group underwent contributed to the development of achievement more than the control group, as it was characterized by endurance exercises for medium and short distances that are directed towards developing oxygen and non-oxygen energy, distances that range between 2 minutes and more are used to improve oxygen endurance, while short distances that range from 15 to 120 seconds are used to develop non-oxygenic endurance. "This is one of the special endurance requirements that an 800-meter running event needs, as it needs both sources of oxygen energy and non-oxygenic, therefore, enduring speed is one of the abilities that may decide the race in the 800-meter event (11), on (Miles, T1992) “and this shows the effectiveness of the exercises included in the curriculum, as it came in line with what happened in terms of achievement, considering that the special endurance is the main factor in the repetition of similar movements such as running
movements according to the endurance of speed and which were included in this achievement test, which was reflected in the results of this group in the post-test as well as the development of the traits included in the program the contestant for this event needs it (12), (Atheer Sabri 1983), citing Yonath, Hack and Carmel, confirms, “The physical qualities of a middle-distance runner are endurance, speed and strength, and these qualities are important and are required to be developed to raise the level of achievement, (Less and more than the racing distance) continuously will inevitably increase the rate of speed over the course of the race and that the development of the speed endurance capacity of the members of the experimental group had the cut-off point in determining the completion time between the two groups as a result of the correlation of the completion time in the 800-meter running event and the variables related to this activity with the development special speed endurance capacity (13).

Table (4) Shows the results of the arithmetic means and the standard deviations of the two research groups in the pre and post-tests in the functional indicators tests.

<table>
<thead>
<tr>
<th>Physical abilities</th>
<th>Test</th>
<th>Measuring unit</th>
<th>Group</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Std. deviation</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Millmole / liter</td>
<td>Experimental</td>
<td>.3612</td>
<td>0.4099</td>
<td>14.840</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>12.66</td>
<td>0.3647</td>
<td>13.420</td>
</tr>
<tr>
<td>VO2max</td>
<td>Milliliter / kg / Minute</td>
<td>Experimental</td>
<td>63.28</td>
<td>2.9132</td>
<td>68.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>61.02</td>
<td>2.4108</td>
<td>62.18</td>
</tr>
</tbody>
</table>

N = 5 for each group.

Table (4) shows in the test of measuring the pulse difference before and after the effort, the mean of the experimental group was lost in the test for measuring lactic acid, so the mean of the experimental group in the pretest was (12.36) and the standard deviation (0.4099), and in the post test the mean became (14.840) and standard deviation (0.7021), as for the mean of the control experimental group, it was (12.66) and the standard deviation (0.3647), and in the post test the mean became (13.420) and the standard deviation (0.249), as for the test to measure the maximum oxygen consumption (VO2max), the mean of the experimental group in the pre-test was (63.28) and the standard deviation (2.9132), and in the post test, the mean was (68.72) and the standard deviation (2.1324), while the mean of the control experimental group was (61.02) and the standard deviation (2.4108), in the post-test, the mean (62.18) and the standard deviation (2.5694) became in order to identify the difference between the pre and post-tests in the results of the physiological indicators tests, the researcher used a T-test for the correlated samples for each of the two research groups.
Table (5) shows the mean of the differences, the deviations of the differences, the value of the (T) test, and the significant significance between the pre and post-tests of the two research groups in the functional indicators tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>Means difference</th>
<th>Std. difference</th>
<th>(T) Calculated</th>
<th>Sig level</th>
<th>Sig Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental</td>
<td>2.48</td>
<td>0.9149</td>
<td>6.061</td>
<td>0.004</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.76</td>
<td>0.4037</td>
<td>4.209</td>
<td>0.014</td>
<td>Sig</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Experimental</td>
<td>5.44</td>
<td>1.7053</td>
<td>7.133</td>
<td>0.002</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.16</td>
<td>0.6768</td>
<td>3.833</td>
<td>0.019</td>
<td>Sig</td>
</tr>
</tbody>
</table>

Degree of freedom (n-1) = 4 and the level of significance (0.05).

Table (5) shows that in the lactic acid measurement test, the mean difference in the mean between the pre and post-tests of the experimental group was (2.48) and the deviation of the differences was (0.9149), and the calculated value of the (T-test) test for correlated samples was (6.061) at a level of significance (0.05), degree of freedom (4), the value of (Sig) was (0.004), which is less than (0.05), which means the significance of the differences between the pre and post-tests in favor of the post test, as for the mean difference of the mean between the pre and post-tests of the control group (0.76) and the deviation of the differences (0.4037), the calculated value of the (T-test) for correlated samples was (4.209) at a level of significance (0.05) and a degree of freedom (4), the value of (Sig) (0.014) is less than (0.05), which means the significance of the differences between the pre and post-tests and in favor of the post test, as for the (VO_{2\text{max}}) test, the mean difference between the two tests of the experimental group was averaged (5.44) and the deviation of the differences (1.7053) and the calculated value of the T-test for correlated samples reached (7.133) with a significance level (0.05) and a degree of freedom (4), and the value of (Sig) (0.002) is less than (0.05), which means the significance of the differences between the pre and post-tests in favor of the post test, as for the mean difference between the pre and post-tests of the control group (1.16) and the deviation of the differences (0.6768) and the calculated value is to test (T-test) for correlated samples (3.833) at a level of significance (0.05) and a degree of freedom (4), the value of (Sig) (0.019) is less than (0.05), which means the significance of the differences between the pre and post-tests in favor of the post-test.
Table (6) shows the mean, standard deviations, \( T \) test value and the level of significance between the post-tests between the experimental and control groups in the functional indicators tests.

<table>
<thead>
<tr>
<th>Functional indicators</th>
<th>Group</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>( T )</th>
<th>Sig level</th>
<th>Sig Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>Experimental</td>
<td>14.840</td>
<td>0.7021</td>
<td>4.262</td>
<td>0.003</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13.420</td>
<td>0.249</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2max</td>
<td>Experimental</td>
<td>68.72</td>
<td>2.1324</td>
<td>4.380</td>
<td>0.002</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>62.18</td>
<td>2.5694</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( N = 10 \) degree of freedom \((n - 2) 5 + 5-2 = 8\) and level of significance \((0.05)\).

Table (6) shows that the arithmetic mean of the experimental group in the post-lactic acid measurement test was \(14.840\) and the standard deviation \(0.7021\), while the control group had its mean \(13\) and the standard deviation \(0.249\), the value of \((T\)-test\) calculated for non-correlated samples was \(4.262\) at a level of significance \(0.05\) and a degree of freedom \(8\), and the value of \((\text{Sig})\) was \(0.003\), which is less than \(0.05\), this means that there is a statistically significant difference with regard to the two research groups and in favor of the experimental group.

Discussing the difference between the results of the pre and post-tests for the two research groups and the \( T \) values of the post-tests between the two experimental and control groups in functional indicators:

From a review of the statistical significance tables \((5 \text{ and } 6)\), physiological indicators (heart rate, lactic acid concentration, and maximum oxygen consumption \(\text{VO}_{2}\max\)) between the pre and post-tests of the two research groups and the post-tests between them, it is evident that there has been an improvement in each of them in the tests and the superiority of the experimental group on control group, the researcher attributes this
result to the proposed exercises that were applied to the experimental group of youth runners, in which repetitions, training stresses and rest periods were codified based on the physical and physiological capabilities of the runners, which were codified through the trainer’s knowledge of the responses firsthand through the time of cutting distances during training. “That a person who has the ability to metabolically adapt is characterized by the ability to work for long periods with low rates of consumption of sources of energy production in the body, that is, the availability of what is known as the economy in the consumption of energy stocks (14), Imad al-Din Abbas states, “The pregnancy given to a player causes excitement and change in the vital organs and systems of the body in terms of function and chemistry, and this appears in the form of improvement in the adequacy of the various organs and systems”, in addition to the distinction of performance, economy by effort, as a result of continuing to perform pregnancy despite the onset of feeling tired and then begins to adapt to this pregnancy (15) and Bastwissi Ahmed mentioned that “the efficiency of muscular work is related to the presence of a large proportion of oxygen in the muscles or its transfer from the lungs to the muscles for movement by means of aerobic and anaerobic reactions (16).

Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached:

4- Speed endurance training have given progress in the level of the (800) meter runners in this test, and they need to be steady and avoid fluctuation in the variations in the values of this test.

5- The results showed the superiority of the experimental group over the control in the physiological indicators (lactic acid concentration, the maximum oxygen consumption (VO_{2max})) in the post-tests.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the General Directorate of Education in Babylon and all experiments were carried out in accordance with approved guidelines.

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Salivary Changes with the Age and their Effect on Plaque Related Disease

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Abstract

Background: Saliva is an exocrine clear oral fluid predominantly secreted by salivary glands both major and minor. It consists of many constituents, mainly water and others are electrolytes, enzymes, immunoglobulins and other antimicrobial factors.

Saliva plays an important role in the oral health, the level of its components change with age, and that has direct effect on teeth and periodontal tissue, because saliva plays a critical role in the development of dental caries and periodontal diseases.

The objective of this study to estimate salivary changes by investigation the level of alpha-amylase and MMP-8 enzymes, sIgA, and salivary minerals (Calcium, Magnesium and Phosphorus) in relation to age.

Materials and Methods: Ninety persons were chosen from different age groups (60) males and (30) females, the age ranged between 20 up to 50, and divided into three subgroups.

ELISA technique was used to evaluate the level of salivary alpha-amylase, MMP-8, and sIgA. Salivary electrolytes were evaluated according to their kits.

Results: Results showed that the level of sIgA significantly decreased with the age, salivary alpha–amylase decreased with the age but not significant statistically, and MMP-8 increased with age but not significant statistically. Regarding salivary minerals (Ca, P, and Mg) results showed that calcium and magnesium levels significantly increased with age, while phosphorus level increased with age also but not significant statistically.

Conclusion: Decrease in the level of sIgA and alpha-amylase with age, while increase in the level of MMP-8 and salivary minerals (Ca, P and Mg) with age, may indicate decrease in the incidence of dental caries and increase in the incidence of periodontal disease with age.

Key words: sIgA; salivary immunoglobulin A, MMP-8; matrix metalloproteinase-8, Alpha amylase enzyme.

Introduction

Saliva is a clear, slightly acidic oral fluid, mostly produced by parotid, submandibular, and sublingual glands and the minor glands. (1) Saliva is an exocrine secretion containing many constituents. It consists mainly of water about 99%, which contains many electrolytes like; calcium, potassium, sodium, magnesium, chloride, bicarbonate, phosphate, and proteins, characterized by immunoglobulins, enzymes, and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides which are of importance to oral health. (2, 3) Saliva plays an important role in the oral health, the level of its components change with age, and that has direct effect on teeth and periodontal tissue, because saliva plays a critical role in the development of tooth decay and periodontal diseases.
Histological analysis; with the advancing age the parenchyma of the salivary glands is replaced gradually by adipose and fibro-vascular tissue, and the capacity of the acini is reduced. Salivary immunoglobulin A (sIgA) is characterized as a part of the immune system “first line of defense” against pathogenic microbes. Restricting the adhesion of microbes, these antibodies respond to biofilm development and thus interfere with the defense of plaque related diseases; dental caries and periodontal diseases. Alpha amylase enzyme considered as one of the main components of saliva that play a role in oral health. In solution, this enzyme, contributing to the bacterial clearance by binding to bacteria. In comparison, it initiates the digestion of starch in enamel pellicle thus give substrates for colonization of bacteria and enhance their adhesion to tooth surface. MMP-8 (matrix metalloproteinase-8) is the most proficient proteinase to initiate degradation of type I collagen and extracellular matrix that associated with the destruction of periodontal and peri-implant tissue, leading to the loss of tooth and dental implant. MMP-8 act as a central mediator in chronic infection, encouraged inflammatory conditions and can exert, anti-inflammatory and defensive properties in addition to the classical surrogate tissue destructive properties. Calcium contributes about 1.9% of the body weight and considered as the most abundant mineral in the body. About (99%) of this percentage in the skeletal system, and the remaining; in the teeth (0.6%), the extracellular fluid (0.6%), the soft tissues (0.6%), and the plasma (0.3%) Calcium provides a structural role in providing rigidity (structure and strength) to the skeleton. Phosphorus considered as the next most abundant mineral in the body after Calcium. These two minerals work together to form strong teeth and bones. Nearby (85%) of the phosphorus in the body is in the teeth and bones. Magnesium regarded as the fourth more abundant mineral in the body. It act as a cofactor for more than 300 enzymatic reactions, where it is essential for the metabolism of adenosine triphosphate (ATP). Magnesium is necessary for the synthesis of DNA and RNA, with protein synthesis.

**Aim of Study**

To study salivary changes according to age by evaluation the level of Alpha-amylase enzyme, MMP-8 enzyme, salivary IgA, and the level of salivary minerals (Phosphorus, Calcium and Magnesium) according to the aging process.

**Material and Methods**

This study was conducted in the center of Hilla city carried out from December 2017 to January 2018. The samples were collected from different age groups from general population. The data were analyzed in University of Babylon \ College of Dentistry

Ninety persons from different ages were chosen: Males (60) and females (30), the age of the persons ranged from 20- up to 50 years. This group of persons are divided into three different age group, as shown in the table below.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>24</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>Group II</td>
<td>22</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>Group III</td>
<td>14</td>
<td>11</td>
<td>25</td>
</tr>
</tbody>
</table>

The saliva was allowed to accumulate in patient’s mouth for two minutes, then the patient was asked to spit the accumulated saliva into the receiving vessel. Two ml of un stimulated saliva samples were centrifuged at 4000 rpm for 15 minutes; the clear supernatant was separated by micropipette and pouted in plane tubes then stored at (-20 °C) in a deep freeze for subsequent analysis which was carried out in maximum period of three weeks. The swab was rotated to remove saliva from the oral cavity, inoculated in media then plate incubated
at (37 °C) for 48 hours anaerobic condition.\(^{(18)}\)

ELISA technique used to evaluate salivary IgA, Alpha- amylase and MMP-8.

Phosphorus according to Phosphorus kit (Phosphomolybdate method) by Mindray, Calcium according to Calcium kit (Arsenazo III method) by using Mindray. Magnesium according to Magnesium Kit (Xylidyl Blue Method) by using Mindray

Statistical Analysis

Data were processed and analyzed with independent Anova- test using statistical package of social science SPSS 19 and the results were expressed as (Mean±SD).P-values < 0.05 were considered statistically significant.\(^{(19)}\)

Results

The study group consists of three different age groups; group I (33) person, group II (32) persons and group III (25) persons, as shown in table (1).

Table (1): Distribution of study samples.

<table>
<thead>
<tr>
<th>Study</th>
<th>NO.</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>33</td>
<td>36.66</td>
</tr>
<tr>
<td>Group II</td>
<td>32</td>
<td>35.55</td>
</tr>
<tr>
<td>Group III</td>
<td>25</td>
<td>27.77</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>100%</td>
</tr>
</tbody>
</table>

Evaluation of salivary IgA

The results showed that Mean ± SD of salivary IgA level for age group I (20-35years) is (492.63±18.30) which is higher than Mean ± SD of age group II (36-50 years) (290.34±22.10) and higher than Mean ± SD of age group III (51 years and above) and the difference among the study groups is significantly decreased with age (P≤0.05). As shown in table (2).

Table (2): Significance of salivary IgA among different age groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Age Group</th>
<th>No. of patients in each group</th>
<th>Mean ± S.D</th>
<th>P. value</th>
<th>Anova Test Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary IgA</td>
<td>20-35 years</td>
<td>33</td>
<td>492.63±18.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36-50 years</td>
<td>32</td>
<td>290.34±22.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51-years and above</td>
<td>25</td>
<td>333.62±36.41</td>
<td>&lt;0.05</td>
<td>*S</td>
</tr>
</tbody>
</table>

*P. value of Anova test≤0.05 was significant.

Evaluation of salivary amylase alpha I enzyme and salivary MMP-8:
Results show that Mean±SD of amylase alpha I enzyme of the age group I is (110.35±66.42), while that of age group II is (71.19±58.53) and of age group III is (85.87±56.62). The difference among the three study groups was decreasing with the age, but not significant statistically (P≥0.05). As shown in table (3).

For the level of MMP-8; in age group I is (0.48±0.50), while in age group II is (0.66±0.85) and in age group III is (0.70±0.80), that shows increasing with age, but not significant statistically (P≥0.05). As shown in table (3).

### Table (3) Significance of Amylase Alpha I, enzyme and MMP8, enzyme among different age groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Age group</th>
<th>Number of patient in each group</th>
<th>Mean ± S.D</th>
<th>P. value</th>
<th>Anova test Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase Alpha I</td>
<td>20-35 Years</td>
<td>33</td>
<td>110.35±66.42</td>
<td>≥0.05</td>
<td>*NS</td>
</tr>
<tr>
<td></td>
<td>36-50 Years</td>
<td>32</td>
<td>71.19±58.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51 Years and above</td>
<td>25</td>
<td>85.87±56.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-8</td>
<td>20-35 Years</td>
<td>33</td>
<td>0.48±0.50</td>
<td>≥0.05</td>
<td>*NS</td>
</tr>
<tr>
<td></td>
<td>36-50 Years</td>
<td>32</td>
<td>0.66±0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51 years and above</td>
<td>25</td>
<td>0.70±0.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P. value of Anova test ≥ 0.05 was no significant.

**Evaluation of salivary minerals (Calcium, Phosphorus and Magnesium) among different age group:**

Table (4) shows that Mean±SD of salivary Calcium for age group I is (6.04±1.87), and for age group II is (5.67±0.79), while for age group III is (8.07±0.56), which shows significant increasing with age (P≤0.05).

For salivary Phosphorus, Mean±SD for age group I is (88.38±16.5) and for age group II is (90.38±5.21), while for age group III is (90.56±17.30) that showed increasing with age but not significant statistically (≥0.05). As shown in table (4).

While Mean±SD of salivary Magnesium level in age group I is (1.94±0.26), for age group II is (1.96±0.38) and for age group III is (2.70±0.44), which shows significant increasing with age (P≤0.05), as shown in table (4).
Table (4) Significance of salivary minerals (calcium, phosphorus and magnesium) among different age group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Age Group</th>
<th>Number of patient in each group</th>
<th>Mean ± S.D</th>
<th>P. value</th>
<th>Anova Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>20-35 Years</td>
<td>33</td>
<td>6.04±1.87</td>
<td>≤0.05</td>
<td>*S</td>
</tr>
<tr>
<td></td>
<td>36-50 years</td>
<td>32</td>
<td>5.67±0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51(Y) and above</td>
<td>25</td>
<td>8.07±0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>20-35 years</td>
<td>33</td>
<td>88.38±16.55</td>
<td>≥0.05</td>
<td>*NS</td>
</tr>
<tr>
<td></td>
<td>36-50 years</td>
<td>32</td>
<td>90.38±5.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51 Years and above</td>
<td>25</td>
<td>90.56±17.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>20-35 years</td>
<td>33</td>
<td>1.94±0.26</td>
<td>≤0.05</td>
<td>*S</td>
</tr>
<tr>
<td></td>
<td>36-50 years</td>
<td>32</td>
<td>1.96±0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51 Years and above</td>
<td>25</td>
<td>2.70±0.44</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

*P. value of Anova test ≤0.05 was significant.

Discussion

Saliva plays an important role in the maintenance of oral health (dental caries and periodontitis). The data of the present study showed that all the participants had dental caries. However, current data expressed a variation in the saliva among different age group. With the age there are some changes occur in salivary flow, amount and composition.

Regarding salivary IgA is a part of the immune system “first-line of defense” against pathogenic microbes, by restricting the adhesion of microorganism.

These antibodies counter to the formation of dental biofilm and therefore interfere with the defense of plaque related pathologies “caries and periodontal diseases” (20, 21, 22). In addition, they act to neutralize enzymes, toxins, and viruses; or by working in cooperation with other factors like lactoferrin and lysozyme. (23)

The results of this study showed that the level of salivary IgA in age group I, is higher than the level of age group II, and higher the level of age group III, and the differences among the study groups show significant decrease with age.

Jafarzadeh, et al., in (2009) and Jafarzadeh, et al., in (2010) found that decline in the salivary IgA levels after the age of sixty years could be attributed to the higher risk of oral infections in the elderly.(24,25)

While Eliasson, et al., in (2006) investigated IgA concentrations in secretions of palatal, buccal, and labial salivary glands in individuals aged 18-72 years. The saliva samples of individuals beyond the age of 65 have shown to have higher salivary IgA levels than other individuals. Increased whole salivary IgA concentrations in older ages have been attributed partly to positive age-related effects on IgA concentrations in the buccal gland secretions. (26)

Childers, et al., in (2003) determined the concentrations of IgA in the parotid saliva of healthy children (age 6-12) and healthy adults (age 22-51) and found that IgA levels increased with the age. (27)

Regarding the concentration of salivary enzyme, the results showed that the concentration of α- amylose I, among different age groups is decreasing with age, but the difference is not significant. These findings are in agreement with that of other studies who establish lesser
α-amylase levels in the elderly (28, 29), while others demonstrated no significant difference, or even

Variations in the results among studies may be due to differences in the methods used, alteration in age groups, and method of saliva collection and saliva used, stimulated or resting type. (30)

There is positive correlation between the level of Ca and α-amylase, because the micro molar levels of Ca2+ are required to stabilize the structure of barley alpha-amyloses in the endoplasmic reticulum of the aleurone layer where these enzymes are synthesized (31). The stabilization mechanism includes an interaction between some negatively charged amino-acid residues and cations. The benefit of this interaction is to keep the three-dimensional structure of protein, which is necessary for the activity of this enzyme. (32, 33)

Elimination of Ca from the genus bacillus, result in reducing thermal stability and enzymatic activity of α-amylase (34), or even increased the susceptibility proteolytic degradation of this enzyme. (35)

Salivary amylase has the ability of metal-binding. Its structure has two sites for metal ion binding, and has one site is selective for Ca binding. It has been found that copper or zinc cannot replace Ca in salivary α-amylase. Therefore, the stability of Ca-amylase binding is a unique interaction. (36)

The presence of Ca in enamel pellicle is necessary for the process of remineralization. While the presence of α-amylase in the enamel pellicle provides the essential substrates for bacterial colonization and enhancing their adhesion to the structure of tooth, and lead to demineralization; therefore, studies about the interaction between Ca and α-amylase in enamel pellicle can provide better explanation for process of remineralization. (36)

Matrix metalloproteinases (MMPs) are family of (24) proteases that play a role in both physiological and pathological conditions. They almost degrade all the components of extracellular matrix and regulate inflammatory processes. They are inhibited by metalloproteinases tissue inhibitors. The main collagenolytic MMP identified in oral fluids is MMP-8, like saliva, oral mouth rinse, gingival crevicular fluid, and peri-implant fluid. MMP-8, that present in oral fluids considered as a strong biomarker that associated with the diagnosis of periodontal disease, their severity, progression, and in fellow-up process. (37)

High level of MMP-8 indicate the loss of supporting periodontal tissues relatively than inflammation. The main collagenolytic MMP detected in the gingival tissue and oral fluids is MMP-8, about (80%) of collagenases found in the gingival crevicular fluid and considered as a periodontal biomarker. (37)

MMP-8 defined as one of the most salivary biomarkers used for detection of alveolar bone destruction that associated with different clinical and radiological parameters, like deepening of periodontal pockets, progression loss of attachment, bleeding on probing and alveolar bone loss. (38)

That explaining the finding of this study, which demonstrated that the level of MMP-8 decreased with age.

Nassar et al., in 2014 found positive correlation between increasing age and salivary MMP-8 levels. These findings were in agreement with the findings of the present study. (39)

A plausible explanation for increased periodontal disease severity with increasing age is prolonged exposure to risk factors over a longer duration and possible influence of undiagnosed concurrent systemic diseases predisposing periodontal breakdown. (39)

Regarding the concentration of salivary minerals, the results showed significant increasing in the level of salivary calcium among different age groups with the age. These findings were in agreement with Sevon et.al, in 2008, who found that salivary Ca levels increased with age (40), while, Salvolini, et al., in 1999 and Chauncey, et al., in 1981, found reduced Ca level in old males. (41, 42)

Phosphorus is the most abundant mineral in the body. Calcium and phosphorus act together to form strong bones and teeth. About (85%) of phosphorus of the body present in the bones and teeth (43). Phosphorus also found in little amounts in tissues and cells all over the body. (43)

The present study showed increasing in the level of salivary Phosphorus with the age. These findings were
in agreement with Sevón, et. al., 2008 who showed that concentration of salivary phosphorus increased with age. (40)

In this study when compared the level of salivary calcium and phosphate in patients with dental caries, periodontitis, and control group, the results showed highly significant statistically, which show high level of calcium and phosphate in patients with periodontitis when compared with controls and dental caries group.

That in agreement with a study done by Sewon et. al., in 1990 who found positive correlation between periodontitis and high level of salivary calcium. (44) Others demonstrated higher concentration of calcium in plaque is associated with low caries incidence. (45, 46)

On the other hand, results showed significant increasing of magnesium level with age.

Both Calcium and Magnesium identified as important elements for the function of different systems in the organisms of human and animal. High level of salivary magnesium, decrease the colonization of streptococcus mutans and therefore, reduced caries possibility. (46)

Gutman and Ben in 1974 demonstrated an elevation in the mean of electrolyte content (Na, K, Ca, and Mg) and a reduction in salivary flow with age; these results indicate probable correlation between the salivary properties and aging process. (47)

The above results explaining the relationship between increased incidence of periodontal disease and decreased incidence of dental caries with the age.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry and all experiments were carried out in accordance with approved guidelines.

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Evaluation of Morphological (Macroscopic and Microscopic) Parameters of Placentas in Pregnancies Complicated by Preeclampsia, Diabetes and Preeclampsia and Diabetes

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1Prof. University of Baghdad, College of Medicine; Department of Anatomy; 2Lect. University of Diyala, College of Medicine, Department of Anatomy

Abstract

This study was done to compare the morphometric parameters of placentas in well controlled patients with preeclampsia, diabetes, and preeclampsia-diabetes with that of normal uncomplicated placentas.

Patients & Methods: A total of Twenty four placentas were freshly collected. Six placentas for control group and eighteen placentas for complicated group (preeclamptic- diabetic and preeclamptic--diabetic subgroups). The placentas were grossly examined (shape, number of cotyledons, weight, and thickness). After suitable fixation, tissue processing and sectioning, the sections were stained by hematoxylin and eosin to study the general morphology and morphometry of the following parameters: number of terminal villi, number of syncytial knots, number of apoptotic cells and number of fetal capillaries.

Result: The weight of the baby in D- subgroup was significantly increased when compared with control group and with P and P-D subgroups . Microscopical morphometry regarding number of terminal villi, apoptotic cells, syncytial knots and fetal capillaries were increased in complicated subgroups. The thickness of basement membrane was 0.8µm in control group and it was increased in all complicated subgroups. The highest value was reported by D- subgroup (2 µm).

Keywords: Parameters, pregnancies, Preeclampsia, Diabetes

Introduction

The placenta acts as a natural barrier between the maternal and fetal blood circulations and fulfills a wide range of endocrine and transport functions. The location between the two bloodstreams makes the placenta not only a crucial regulator of fetal nutrition, gas exchange, and maternal immune tolerance, but makes this fetomaternal organ also a target for maternal and/or fetal metabolic alterations associated with pregnancy pathologies. One of these pregnancy pathologies is gestational diabetes mellitus (GDM)(1). DM is a common clinical condition that affects approximately 1 to 15% of pregnant women(2). GDM is a metabolic disease defined as progressively impaired glucose intolerance with the onset or first recognition during pregnancy(3). Fetuses with intrauterine exposure to hyperglycemia more often present with macrosomia, birth trauma, neonatal hypoglycemia, and respiratory distress syndrome (4). The second pregnancy pathology that affects the placenta is preeclampsia which is a hypertensive disorder of pregnancy defined by the American College of Obstetrics and Gynecology (ACOG) as 'new onset of hypertension on two separate occasions with proteinuria arising de nova after the 20th weeks of pregnancy in a previously normotensive non-proteinuric woman(5). Pre-eclampsia is a human pregnancy-specific disorder with an incidence of 2-8% worldwide (6). The incidence being 3 to 7 % in nulliparous and 1 to 3 % in multiparous. It is directly associated with 10 to 15 % of maternal deaths(7). The diagnostic criteria for preeclampsia is systolic blood pressure of >140mm Hg or diastolic blood pressure of >90mm Hg measured at rest on two different occasions at least six hours apart accompanied by proteinuria of >0.3 g in a 24-hour urine specimen (8).
The placenta is a mirror which reflects the intrauterine status of the fetus. An adequate knowledge of the morphometric analysis of the placenta proves to be useful in the early assessment of placental sufficiency and also the state of fetal wellbeing. Hence, this study was done to compare the morphometric parameters of placentas in well controlled patients with pre eclampsia, diabetes, and preeclampsia-diabetes with that of normal uncomplicated placentas.

**Materials & Methods**

A total number of 40 freshly delivered placentas were collected from the government hospital for women and children in Diyala. The placentas were collected soon after their expulsion, from normal deliveries. They were divided into two major groups; control group (10 women) and complicated group (30 women). The latter was divided into three subgroups (10 women each), pregnant women with pre eclampsia(P); pregnant women with diabetes(D) and pregnant women complicated by both pre eclipampsia and diabetes(P-D). Clinical criteria for selection of patients were shown in table 1. The collected placentas were washed under running tap water and the membranes were thoroughly examined and trimmed. The umbilical cord was cut, leaving a length of 5cms from its placental site of insertion.

The specimens were then transported in formalin (10%) filled plastic containers to the Department of Anatomy.

In all the collected placentas, the following parameters were studied: weight, shape, placental thickness and the number of cotyledons. From each placenta, tissue pieces measuring 0.25×0.25 cm were taken 2 cm from the attachment of umbilical cord. After 24 hours fixation in 10% formalin (Fluka AG. Chemicals, Buchs), the fixed tissues were processed for routine paraffin- wax embedding. From each paraffin tissue block, 2 sets of sections were prepared. The 1st set was used for routine heamatoxylin - eosin stain; the 2nd set for the Periodic Acid Schiff’s reaction (PAS). In our study microscopic morphometry included the following parameters: number of apoptotic cells, number of terminal villi, number of syncytial knots, number of fetal capillaries and thickness of trophoblastic basement membrane (microns) using 40X objective and 10X ocular at 6 random fields per sections.

The data collected were analyzed using the computer facility with the available software statistical packages of SPSS 17 (Statistical Packages for Social Science, version 17.0). Results were presented in simple measure of mean ± S.D.(standard deviation). The significance of difference among quantitative variables of groups was assessed using one-way analysis of variance (ANOVA).

**Results**

In all groups, the placenta is disk like, and round to oval. The fetal surface appeared congested in P-subgroup, ranged from red-purple to shiny translucent in D- and D-P subgroups. Subchorionic fibrin/fibrinoid deposits and infarcted area were significantly increased in all complicated subgroups (table 2).

- Macroscopic parameters of the placenta (Table 3 and 4):

  - The number of cotyledons were significantly decreased in all complicated subgroups. The weight of the placenta in control group was 510 gm. In P subgroup was significantly decreased (375.8gm) and in D- and D-P subgroups were non significantly increased and they were 550gm and 521.6gm respectively.

  - The weight of the baby in control group was (3191gm). In D- subgroup was significantly increased (4300gm) when compared with control group and with P and P-D subgroups.

  - The thickness of placenta was 2.5 cm in control group while in all complicated subgroups, the thickness of placenta was 2.2cm

- Microscopic Examination of Placenta (Tables 5 & 6):

  - The number of villi in control group was 10.5 and it was significantly increased in P, D and P-D subgroups when compared with control group.

  - The number of apoptotic cells in control group was 11.4 and it was significantly increased in P, D and P-D subgroups when compared with control group.

  - The number of syncytial knots were 12.4 in control group and their number was significantly increased in P, D & P-D.
subgroups when compared with control group.

The thickness of basement membrane was 0.8µm in control group and it was increased in all complicated subgroups. The highest value was reported by D-subgroup (2 µm).

The number of fetal capillaries was 8.3 in control group. It was significantly decreased in P– subgroup when compared with control groups and with D-and P-D subgroups and was significantly increased in D- and P-D subgroups when compared with control and P-groups.

Discussion

Round or oval placentas are the predominant human placental form, but many other shapes exist (Bilobed placenta, Placenta membranacea, Succenturiate placenta, Fenestrated placenta, Ring (zonary) placenta (12). Anomalies may develop from abnormal fetal genes expressed by the placenta, an abnormal maternal environment, or an abnormal fetal-maternal interaction (13). As all the subjects were apparently healthy and there was no evidence of maternal malnutrition. The hemoglobin level was about 10gm/ dl in all subjects included in this study. This may be the reason of normal shape. Only in severe malnutrition, abnormal shape has been reported by previous workers (14,15).

In our study, fibrinoid deposits moderately increased in all complicated groups which mean that focal degeneration of syncytiotrophoblast increased in these groups. This finding is in agreement with older findings by Horman who considered that factors such as hypoxia and acidosis might lead to syncytial degeneration and eventually result in replacement of the syncytiut by fibrinoid(16).

The number of cotyledons, in complicated group, was significantly decreased. This may be explained by an altered distribution of fetal blood in complicated placenta resulting in different modes of arrangement of intracotyledonary vessels of complicated pregnancy(17).

The placental weight was significantly decreased in preeclampsia. This finding corroborate with the study of Fox (18). The placental weight in D and P-D subgroups was non significantly increased as it had been found by Clarson et al. that when diabetes is well controlled during pregnancy, the placental weight does not deviate from that of normal organs (19).

This study reveals that the fetal weight was significantly decreased in P-subgroup. This finding corroborate with the studies of other workers (18,19,20). Rath stated that in hypertension; arrangement of the intracotyledonous vasculature is altered resulting in low birth weight of babies (17). The birth weight in P-D and D subgroups was increased and it was significant in the latter. The weight gain in diabetic’s placentas may be attributed to macrosomia and compensatory hyperplasia. Macrosomia affects the fetus and fetal part of placenta i.e. chorionic plate. The macrosomia may be attributed to fetal hyperinsulinemia in response to hyperglycemia in fetuses of diabetic mothers (21).

Thickness of the placenta depends upon the length of the villi (22). In present study, the thickness of placenta (length of stem villi) in all complicated subgroups was non significantly decreased when compared with control group.

Longitudinal growth of the capillaries within the mature intermediate villi exceeds that of the villi themselves so the capillaries coil and form loops that bulge from the villous surface, forming grape-like outgrowths known as terminal villi (23). The development of this low impedance capillary network parallels the proportional rise in fetal cardiac output entering umbilical arteries to about 40% at term (24). In our study, the number of terminal villi was significantly increased in all complicated subgroups and this means that the capillary growth exceeds the longitudinal villous growth in these groups which may occur secondary to an increase of fetal cardiac output entering umbilical arteries in these groups.

Apoptosis is initiated via the extrinsic or intrinsic pathway. Both pathways rely upon a cascade of protein interactions orchestrated by a family of 14 cysteine proteases, caspsases, which are able to cleave structural proteins producing the morphological appearances typical of apoptosis. In addition, active caspsases potentiate the apoptotic signal by activating a variety of pro-apoptotic proteins (25). The extrinsic pathway is controlled by members of the tumour necrosis factor (TNF) death receptor family (26). The intrinsic pathway is initiated by cellular stress; such as DNA damage, reactive oxygen species, the unfolded protein response,
or removal of growth factor support\textsuperscript{(27)}. In our study, the significant increase in apoptotic cells in P- and D- subgroups may be attributed to an exaggeration of extrinsic and/or intrinsic pathways.

Syncytial knots are consistently present, increasing with increasing gestational age, and can be used to evaluate villous maturity. Increased syncytial knots are associated with conditions of uteroplacental malperfusion and are important in placental examination\textsuperscript{(28)}. Therefore, the significant increase of syncytial knots in P- and D-subgroups was due to fetal malperfusion of placental villi.

It is well known that molecular composition of the basement membrane changes during maturation of villous trophoblastic and endothelial basement membranes, these changes are particularly obvious in area of trophoblast proliferation and villous sprouting\textsuperscript{(29)}. Due to increased incidence of apoptosis, large number of parenchymal cells (trophoblast, endothelial cells) has been observed to be eliminated and replaced by fibrous tissue\textsuperscript{(30)}. This fibrous tissue was synthesized by fibroblasts of villous stroma. Fibroblasts also take part in the synthesis of subtrophoblastic basement membrane\textsuperscript{(31)}. In this way, secondary to increase incidence of apoptosis in all complicated subgroups, large number of parenchymal cells (trophoblast, endothelial cells) had been replaced by fibrous tissue and this may play a role in increase thickness of basement membrane.

Placental angiogenesis can be subdivided regarding its mechanisms and the geometry of the resulting vascular bed\textsuperscript{(32,33,34)} into branching angiogenesis (multiple sprouting of micro vessels produces a complex multiply branched capillary web) and non-branching angiogenesis(Vascular bed expands by elongation of existing capillary loops). In this way, the significant decrease in number of fetal capillaries in P- subgroup may be due to non-branched angiogenesis which may become the only mode of angiogenesis which is suppressed by binding of PI GF to VEGFR-1. The significant increase of fetal capillaries in D-and P-D subgroups has occurred as branched angiogenesis may become the only mode of angiogenesis which may be stimulated by binding of VEGF-A to its receptors.

<table>
<thead>
<tr>
<th>Table 1 Shows clinical criteria for control and complicated groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>P*</td>
</tr>
<tr>
<td>D*</td>
</tr>
<tr>
<td>P-D*</td>
</tr>
</tbody>
</table>

*Preeclamptic patients were treated with aldomet, Diabetic patients were treated with insulin and preeclamptic –diabetic patients treated with aldomet and insulin.
Table 2 shows deposition of fibrin/fibrinoid in control and complicated groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fibrin/Fibrinoid (numbers of patches)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt;10</td>
</tr>
<tr>
<td>P</td>
<td>&gt;20</td>
</tr>
<tr>
<td>D</td>
<td>&gt;20</td>
</tr>
<tr>
<td>P-D</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

Subchorionic fibrin/Fibrinoid per/fetal surface area

Table (3) shows Mean ± standard deviation of macroscopical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Complicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>Number of cotyledons</td>
<td>38</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Weight of Placenta/gm.</td>
<td>510</td>
<td>375.8</td>
</tr>
<tr>
<td></td>
<td>52.1</td>
<td>24.1</td>
</tr>
<tr>
<td>Thickness of placenta/cm</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Weight of Baby/gm.</td>
<td>3191.6</td>
<td>3158.3</td>
</tr>
<tr>
<td></td>
<td>162.5</td>
<td>638.2</td>
</tr>
</tbody>
</table>

Table (4) shows Multiple comparison(ANOVA) of macroscopic parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>P</th>
<th>D</th>
<th>P-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of placenta</td>
<td>Control</td>
<td>0.09*</td>
<td>0.00*</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.009*</td>
<td>0.246</td>
<td>0.738</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.000*</td>
<td>0.406</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>P-D</td>
<td>0.004*</td>
<td>0.738</td>
<td>0.406</td>
</tr>
<tr>
<td>Weight of the baby</td>
<td>Control</td>
<td>0.863</td>
<td>0.000*</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.863</td>
<td>0.000*</td>
<td>0.043*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>P-D</td>
<td>0.063</td>
<td>0.043*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Number of cotyledons</td>
<td>Control</td>
<td>0.000*</td>
<td>0.024*</td>
<td>0.010*</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.000*</td>
<td>0.130</td>
<td>0.247</td>
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<td></td>
<td>D</td>
<td>0.024*</td>
<td>0.130</td>
<td>0.712</td>
</tr>
<tr>
<td></td>
<td>P-D</td>
<td>0.010*</td>
<td>0.247</td>
<td>0.712</td>
</tr>
<tr>
<td>Thickness of placenta</td>
<td>Control</td>
<td>0.332</td>
<td>0.363</td>
<td>0.363</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.332</td>
<td>0.951</td>
<td>0.951</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.363</td>
<td>0.951</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>P-D</td>
<td>0.363</td>
<td>0.951</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Table (5) shows Mean ± Standard deviation of microscopic parameters

*The mean difference is significant at the 0.05 level.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Complicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>Number of villi</td>
<td>10.5</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>1.15</td>
<td>1.4</td>
</tr>
<tr>
<td>Number Apoptotic cells</td>
<td>11.4</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Number of Syncytial Knots</td>
<td>12.4</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Thickness of basement membrane/µm</td>
<td>0.8</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>8.5E-02</td>
<td>6.7E-02</td>
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<tr>
<td>Number of fetal Capillaries</td>
<td>8.3</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0.3</td>
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</table>

Table (6) shows Multiple comparison (ANOVA) of microscopic parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>P</th>
<th>D</th>
<th>P-D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>D</td>
<td>P-D</td>
<td></td>
</tr>
<tr>
<td>Number of villi</td>
<td>Control</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.017*</td>
</tr>
<tr>
<td></td>
<td>0.360</td>
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<tr>
<td></td>
<td>0.046*</td>
<td></td>
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<tr>
<td></td>
<td>0.016*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of apoptotic cells</td>
<td>Control</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>0.551</td>
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<tr>
<td></td>
<td>0.029*</td>
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<td></td>
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<tr>
<td></td>
<td>0.015*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of syncytial knots</td>
<td>Control</td>
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<td>0.000*</td>
<td>0.035*</td>
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<td></td>
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<td></td>
<td>0.342</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.406</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness of basement membrane</td>
<td>Control</td>
<td>0.894</td>
<td>0.879</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>0.051</td>
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<td>0.982</td>
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<td></td>
<td>0.978</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fetal capillary</td>
<td>Control</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>0.011*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.000*</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
*The mean difference is significant at the 0.05 level.

**Conclusions**

An adequate knowledge of the morphometric analysis of the placenta with its clinical relevance proves to be useful in the early assessment of placental sufficiency and also the state of the fetal wellbeing. In mothers who have had no previous antenatal checkup, a thorough examination of the placenta helps in the early diagnosis of the fetal complications, soon after parturition and thus helps in the early treatment of the baby by neonatologists.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College Of Medicine and all experiments were carried out in accordance with approved guidelines.

**References**

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Parenting Styles and Children’s Behaviors Difficulties

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1Assistant. Lect., Pediatric Health Nursing, College of Nursing, University of Babylon, 2M.B.Ch.B._F.I.C.M.S (Pediatrics), 3Assistant. Prof., Pediatric Health Nursing, College of Nursing, University of Babylon

Abstract

The descriptive-correlation study design used to investigate the relation between parenting styles and children’s behavior difficulties by using the Parenting Styles and Dimensions Questionnaire, and Strengths and Difficulties Questionnaire to achieve study objectives. An online questionnaire applied as a method of data collecting on 101 parents (majority of them mothers) and their 4-11 years old children, 58 of them were males and 43 were females. The finding of the current study reveals that the authoritative parenting style was used more than other styles and correlated significantly with good child behaviors while the authoritarian and permissive style used less and correlated negatively with child’s behaviors. In conclusion, when parents use an authoritative or democratic style enhances children’s positive behaviors more than other styles.

Keywords: Parenting styles, behavior’s problems, and behavior’s difficulties

Introduction

Parenting style characterizes as emotional relationship and the quality of parent-child interaction, and this interaction has great importance in the learning, development process, and behaviors of a child(1).

Diana Baumrind at the end of the 20th century categorized parents based on two dimensions; responsiveness and demandingness, and describe three different kinds of parenting styles: (authoritative, authoritarian, and permissive). Authoritative parents have a high level of responsiveness and demandingness to their children but being firm in setting rules and limits. They direct their children’s behaviors and attitudes by emphasizing the reason for rules and by negative reinforcement(2).

Authoritarian parents have low responsiveness and high demandingness for absolute obedience. They try to control their children’s behaviors and attitudes through unquestioned mandates. They establish rules and regulations or standards of conduct that they expect to be followed rigidly and unquestioningly(3).

The permissive parenting style is characterized by high responsiveness and low demandingness. They use little or no control over their children’s actions. They avoid imposing their standards of conduct and allow their children to regulate their activity as much as possible. These parents consider themselves to be resources for the children, not role models.

Many previous studies show that the children’s behaviors earned in the early years shape their personality structure, attitudes, beliefs, and value judgments of individuals. Children acquired most of these behaviors from their parents, which consider the first teacher(4).

The interaction between parents and children have a great effect on their social development and behaviors, and across the years and these interactions diminished because of many factors; one of them was the occupied parents with digital devices or technologies(5,6).

Parents may use one or more than one style or may change the parenting style as the child grow up. In other words, if the parents fall in choosing the proper parenting style or use inappropriate behaviors when dealing with their children this can affect children’s socialization. Therefore, knowing a child’s behaviors, characteristics, and temperament by their parents help them to use or adjust the appropriate parenting style(7).
The present study aimed to investigate child behavior difficulties and to find out the association between parenting style and children’s behavior difficulties.

**Method**

**Design & Setting of the study:** A descriptive correlational study design was used in this study throughout the period 1st of February 2020 to the 3rd of May 2020.

At the beginning of the outbreak of COVID-19 in Iraq, the government took many restrictive measures to decrease the spread of contagion one of them was a curfew; during the period of quarantine, the family members stay at home and avoid crowding to protect themselves from getting a disease (infection). Therefore the researchers used an online questionnaire as a method for data collection in this circumstance; the online questionnaire was answered in March of 2020 by 101 parents of children age (4-11 years old) live in the Babylon governorate. The questionnaire consisted of three parts:

1. Demographical characteristics of parents and their children 4-11 years old (child age, child gender, parent (father or mother), parents’ educational attainment, parents’ occupation, family income, and numbers of family members.

2. The Parenting Styles and Dimensions Questionnaire (PSDQ)\(^8\) was used to identify the preferable parenting style by Babylonian’s parents and it is extracted from (9). PSDQ consisting of 30 items (13 for authoritative style, 13 for authoritarian style, and 4 items for permissive style), each item rated on a scale of 1 (*never*) to 3 (*always*). All three categories of parenting style demonstrated good internal consistency (Cronbach’s alpha was reported 0.77 for authoritative, 0.78 for authoritarian, and 0.72 for permissive).

3. Strengths and Difficulties Questionnaire (SDQ)\(^10\). SDQ is a 25-items parent or teacher-form measure of children’s behaviors from 4-17 years old. This form is a 3-level Likert scale, scored as (0 = doesn’t apply, 1 = apply sometimes and 2 = certainly apply). Some items were reversed for the statistical purpose. The rate of the scale was based on the mean of items’ scores to (0 - 0.66) very poor behaviors, (0.67 - 1.33) poor behaviors, and (1.34 - 2) good behaviors. Good reliability and validity of the SDQ have been reported in a previous study\(^11\).

**Statistical Analysis:** Data was analyzed by using SPSS (statistical package for social sciences) version 20 to find out the associations between parenting styles and child behavior difficulties by use personal correlational coefficient and use the descriptive statistic for demographical variables and to identify the preferable parenting style.

### Results

**Table (1) Characteristics of Children and Their Respondent’s Parents (N = 101)**

<table>
<thead>
<tr>
<th>Children’ Characteristics</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preschool</td>
<td>57</td>
<td>56.5</td>
</tr>
<tr>
<td>School Age</td>
<td>44</td>
<td>43.5</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58</td>
<td>57.4</td>
</tr>
<tr>
<td>Female</td>
<td>43</td>
<td>42.6</td>
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</tbody>
</table>
Table (1) Characteristics of Children and Their Respondent’s Parents (N = 101)

<table>
<thead>
<tr>
<th>Child order</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
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<td>13.9</td>
<td>18.8</td>
<td>10.9</td>
<td>8.9</td>
<td>11.9</td>
</tr>
</tbody>
</table>

| Characteristics of the respondent |
| F | % |

| Parents | Father | 15 | 14.9 |
|         | Mother | 86 | 85.1 |

<table>
<thead>
<tr>
<th>Educational attainment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read and write</td>
</tr>
<tr>
<td>Primary</td>
</tr>
<tr>
<td>secondary</td>
</tr>
<tr>
<td>Diploma and above</td>
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</table>

<table>
<thead>
<tr>
<th>Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working</td>
</tr>
<tr>
<td>Not working</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Family income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-Sufficient</td>
</tr>
<tr>
<td>Sufficient to some extent</td>
</tr>
<tr>
<td>Sufficient and more</td>
</tr>
</tbody>
</table>

Table (1) show that the total children were 101 divided into 56.5% preschoolers and 43.5% school-age children; regarding their gender more than half was male and the 1st order was the higher percent 35.6% in the family. The majority of the participant from parents were mothers, in addition to their educational attainment was diploma and above, two-thirds of them were not working and most of them say that their income was sufficient to some extent.
Table (2) preferable parenting style

<table>
<thead>
<tr>
<th>Parenting style</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authoritative</td>
<td>1.46</td>
<td>3</td>
<td>2.48</td>
<td>0.266</td>
</tr>
<tr>
<td>Authoritarian</td>
<td>1.31</td>
<td>2.77</td>
<td>1.97</td>
<td>0.310</td>
</tr>
<tr>
<td>Permissive</td>
<td>1.25</td>
<td>2.75</td>
<td>1.85</td>
<td>0.357</td>
</tr>
</tbody>
</table>

Descriptive statistics used in this table to identify the preferable parenting style of Babylon’s parents. Authoritative parent style was more preferred by parents according to the finding.

Figure 1 Children behaviors difficulties

Table (3) correlation between parenting style and child behaviors

<table>
<thead>
<tr>
<th>Children’s Behaviors</th>
<th>Authoritative</th>
<th>Authoritarian</th>
<th>Permissive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation</td>
<td>.369**</td>
<td>-.336-**</td>
<td>-.165-*</td>
</tr>
<tr>
<td>Sig. (1-tailed)</td>
<td>.0001</td>
<td>.0002</td>
<td>.0497</td>
</tr>
<tr>
<td>N</td>
<td>101</td>
<td>101</td>
<td>101</td>
</tr>
</tbody>
</table>

*. Significant at (P<0.05), **. Significant at (P<0.01)

Bivariate (Pearson) correlates analysis was used to identify the association between a child’s behavior difficulties and type of parenting style. The finding showing a positive- significant correlation between children’s behaviors and the authoritative parenting style, which means when the parents use this style their child behaves in a good way. On the contrary, the other parenting style (authoritarian and permissive) showing a significant negative correlation with children’s behaviors. This means when these styles are always used by a parent the child exhibits poor or difficult behaviors.
Discussion and Conclusion

The aim of the current study was to find-out the association between experienced parenting styles and behavior difficulties in preschool and school-age children in the Babylon governorate. The current findings revealed that the Authoritative parenting style was preferred or used more than others. The authoritative or democratic parenting style was most commonly used by Babylonian’s parents and that may due to their benefit in improving the parent-child relationship and enhancing good communication and interaction between them, besides aiding in the proliferation of child’s creativity (1) as well as raising social responsibility and a sense of self-esteem of the child as the previous study has shown (12). Moreover, The result of the current study goes in line with the Jordanian study conducted by and Lo et al., (3) study.

The results in Table (3) showed a positive-significant correlation between children’s behaviors and authoritative parenting style while, the others parenting style (authoritarian and permissive) showing a significant negative correlation with children’s behaviors. This was parallel with (14) study and similar to the (15) study, which mentions that the family whose use authoritative style; their children demonstrate a lower level of problematic behavior, greater academic competence, and a higher level of psychosocial development than children who come from authoritarian, permissive and neglecting families. Furthermore, a parent that practicing authoritarian style was significantly associated with child abuse, and that lead to increase stress and affect negatively on family climate (16,17). In contrast with the parent who used authoritative style was associated with a lower risk of all types of child maltreatment (physical abuse, psychological abuse, and neglect) (3). In conclusion, the Babylonian parents practicing authoritative style more than other styles, and when they use the authoritative or democratic style enhance their children’s positive behaviors more than other styles.

Summary:

This study showed that the authoritative parenting style was used more than authoritarian and permissive styles by Babylonian parents and there is a significant correlation between authoritative parenting style and good child behaviors, while there is a significant correlation between authoritarian and permissive styles and negative child’s behaviors. This means when the parent uses authoritarian and permissive style the children exhibit behavior difficulties more than those children of a parent that use authoritative style.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


17. Cantero-Garcia M, Alonso-Tapia J. Evaluación del clima familiar creado por la gestión de los problemas de conducta, desde la perspectiva de los hijos. 2017;
Assessment of Children with Thumb Sucking Habit in Babylon City

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Abstract

Aims: The purpose of this study is to assess the prevalence of thumb sucking habit between preschoolers and the effect on their occlusion of the habit.

Materials and Methods: 42 of pre-school children aged 4-5 years who were inspected by questioning for the presence or absence of thumb sucking habit, and thumb examination, and the teeth were examined for the presence of decreased over flight and overbite or open bite.

Results: Total number children have thumb sucking habit, and open bite of them.

Conclusion: In this age group (4-5) years, the number of children with thumb sucking habit was considered high and the habit was found to increase the prevalence of malocclusion of deciduous.

Keywords: Thumb, Children, Babylon, school.

Introduction

The incidence of malocclusion is greater in children with sucking habits than in children with no habit at the age of 3-12, but when children avoid sucking their fingers before the age of 6, they do not have a higher rate of malocclusion than children with no history of sucking habits(1,2).

1. Oral habits should be of paramount health interest to orthodontists because they can induce malocclusion and interfere with the advancement of therapy (4). Irregular oral habits work so silently and unintentionally that even the patient is often unaware of their presence. Originally, all these basic habits are performed by deliberate attention, with each occurrence being less and less conscious effort.

Sucking habit is the infant’s fist-coordinated muscle activity and is also considered a natural reflex. There are essentially two forms of sucking; the nutritional form that provides essential nutrients, while non-nutritional sucking provides a feeling of warmth and a sense of safety, and this type of sucking can be considered as an important first step in the child’s self-regulation development.

So that, babies suck to comfort themselves when they are upset, it’s also away for exploring and in some cases, sucking may be just away for babies to pass the time (9,10), but many children recognize that the habit is an infantile mechanism, they find it difficult to leave because it becomes an enjoyable habit by the time (11). Besides, they may use it to get their parents’ attention (12,13).

The sucking habit period is important, a short period of time may have no or little effect (13,14). In some children, the sucking habit is little or more than a passive insertion of the finger into the mouth, with no apparent buccinators activity (14,15), so that children who suck vigorously but intermittently may not displace the incisors much, if at all, while others who produce 6 hours or more may not displace them.

Literature Review

A review of the literature on thumb sucking habit prevalence shows that this varies from one population to another. The aim of this research was: 1) to evaluate the prevalence of sucking habits among Iraqi pre-school
children living in Babylon City; 2) to assess the impact of certain cultural factors on that prevalence; and 3) to study the effect that these habits could have on primary dentition. Cross-sectional

Study was conducted using a survey questionnaire and clinical examination of 42 Iraqi children aged 3-5 using a stratified random sampling technique for clusters. The prevalence of sucking habits with the dummy-sucking being the dominant type was 48.36 per cent. In the first few years of life most dummy suckers had lost their habits while more digit-suckers were still present at age 5. Sucking behaviors were related only to the schooling of the parents and the methods of feeding the infant without any major influence of gender or birth rank or family income. Kids with current digit sucking habits had slightly more distal molar and canine relationships of Class II (P < 0.05), greater over jet, and wider bite than kids without sucking habits.

Such variations became also more important (P < 0.01) when compared dummy-suckers to non-suckers. A more flexible snap was the only observable consequence of past sucking habits. In children with sucking habits the posterior cross bite was no more common than in children without these habits. The concern regarding sucking habits is evident from the number of articles that have appeared in scientific journals over the past 50 years.

Methodology

The sample of this study was consisted of 50 preschool children from districts in Babylon city, with age range of 4-5, the selection of this age group is to reveal the prevalence and effect of thumb sucking habit before the time of eruption of permanent teeth. The children were divided into two groups, first group consisting children with thumb sucking habit and the second consisting children without thumb sucking habit. the children were examined for the presence of thumb sucking habit ;the sucked digit often reveal callous formation and under development (16) in addition to that a history is taken from the teaching staff about the presence of the habit for all children to confirm the clinical findings, the overjet was measured by avernia by measuring the distance between the labial surface of lower central incisors and labial surface of upper central incisors ,while the overbite is measured by considering the amount of vertical coverage of the upper central incisors to the lower central incisors.

Results

<table>
<thead>
<tr>
<th>Habits</th>
<th>Boys (N=25)</th>
<th>Girls(N=17)</th>
<th>Total(N=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Dummy</td>
<td>18</td>
<td>36</td>
<td>9</td>
</tr>
<tr>
<td>Digit</td>
<td>7</td>
<td>5.4</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>69.4</td>
<td>17</td>
</tr>
</tbody>
</table>

Table(1) Show that the (42) examined children (15)of them have thumb sucking habit (9%) of the total

Discussion and Conclusion

Prevalence of thumb sucking habit in preschool children and its effect on deciduous teeth was not adequately dealt with in researches; on the other hand, it may be much easier to manage the habit at early stages than to leave it to become more enjoyable behavior to the child, so that data of prevalence and effect of T.S.H. during this stage of child’s life is beneficial to know how much efforts are needed to assist children to leave the habit at early stages. This study found that the percent
of preschool children having T.S.H. is 9% which is not angelic table percent because part of them may retain the habit causing malocclusion of the permanent teeth. This study also made accompanist of dental occlusion between children with and without T.S.H.

It was found that the percent of children having increased over jet is higher in the group having T.S.H. which may be due to proclanation of maxillary incisors and retroclination of mandibular incisors; and the overbite in the group having T.S.H. is higher which may be due to over eruption of upper incisors due to the increase in the over jet, and the percent of children with open bite in the group with T.S.H. is higher which may be due to a combination of interference with normal eruption of incisors, and excess eruption of posterior teeth, these findings agree with the findings obt the differences between the two groups in all types of changes in occlusion (overjet; overbite) are statistically non-significant which may be due to the small percent of children with increased overjet, overbite and open bite in the control group due to causes other than T.S.H, like; class 2 malocclusion, mouth breathing, tongue thrusting.

It can be concluded that thumb sucking habit effect the deciduous teeth by increasing over jet, overbite and prevalence of open bite; these effects may arise in permanent teeth if the habit was retained, and the percent of children having the habit of thumb sucking at this age range is still considered to be high, so it is suggested that parents should be instructed to consult psychiatrist and orthodontist if the child still having the habit at this age, to stop the habit as soon as possible.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Stakeholders’ Perspectives on Quality of Death Registration
And Possibility of Introducing Verbal Autopsy Into Kurdistan Region of Iraq

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Abstract

Aim: To explore stakeholders’ perspectives on the quality of death registration and possibility of introducing verbal autopsy into the Kurdistan Region, Iraq.

Methods: Qualitative interviews conducted with 15 stakeholders from the Ministry of Health, Directorates of Health, and Medico-legal Institutes of Erbil, Sulaymaniyah, and Duhok of the Kurdistan Region of Iraq. Stakeholders were selected through the purposeful sampling method. Responses were analyzed using qualitative content analysis and thematic analysis. Responses were categorized into three main categories: strengths, gaps, and suggestions for improving the death registration in the Kurdistan Region.

Results: The strong points in favor of the current death registration were: easy and good practice, it was easy for people to get a certificate, the existence of laws and regulations related to death registration, having a “standard death certificate,” and follow-up and monitoring of the process of death registration at the Medico-legal Institutes. The gaps were incompleteness and inaccuracy in most of the locations, staff ignorance, no checking of data, missing cases, no use of the data, and no standardized process for compiling data.

The stakeholders emphasized on implementing the existing rules and regulations, building staff capacity, systematic processes for data management, and follow-up and monitoring as strategies for improvement.

All the stakeholders reported that verbal autopsy is appropriate for solving problems related to cause-of-death data. However, the feasibility of verbal autopsy will be a challenge for several reasons, including the current economic crisis.

Keywords: Death registration, Kurdistan Region of Iraq, verbal autopsy.

Introduction

Death registration is a key element for health system planning, evaluating the effectiveness of health services, and epidemiological studies. However, inaccuracies in death registrations are a global problem and many reports on death statistics from countries to the World Health Organization (WHO) have incomplete or inaccurate death registrations. Even in developing countries, a significant proportion of deaths occur without medical information on the cause of death. In order to support policy development, death registration should have all detailed records of the decedents’ ages, sex, and correct causes of death. Death registration in the Kurdistan Region of Iraq is produced through three ways:

• Death that occurs in hospitals: the attending senior physician specifies the cause of death and the
registrar issues a “death certificate.”

- Death from natural causes that occurs at home: the relatives of the deceased person report to the court and get a “legal death certificate” that has no information on the cause of death.

- Death from unnatural causes, e.g., due to accidents: the medico-legal institutes investigate the cause of death and issue a death certificate.

The system for death registration in Iraq is based on records from hospitals and is mainly administrated manually. The study of Zangana et al. in the Kurdistan Region revealed inaccuracies in the cause of death at registration and limited use of death certificates. They added that there was an urgent need for educational efforts to achieve a complete and accurate death registration.

Method

Setting

This study was carried out from July 2017 through May 2018 in three governorates of the Kurdistan Region of Iraq: Erbil, Sulaymaniyah, and Dohok. The study participants were from all the sites that deal with death registration in order to obtain the perspectives from all the jurisdiction levels namely the Ministry of Health (MoH), the Directorates of Health (DoH) and the Medico-legal Institutes (MLI) of all three governorates of Kurdistan region of Iraq. A total 15 stakeholders were interviewed; two were from the MoH (13.3%), seven were from the DoH (46.6%) and six were from the MLI (40%). Participants included males and females. The participants were selected, via the purposeful sampling method, because of their knowledge and familiarity with death registration.

Instruments

The researchers developed a set of semi-structured qualitative interview guides that consisted of open-ended questions with specific follow-up probes. The qualitative data collection tool was in Kurdish language and then translated into English language with adjustments to phrasing.

The qualitative interview explored the participants’ perspectives about the current death registration practice, particularly regarding its strengths and gaps in effectiveness and suggestions for improvement. Moreover, specific thoughts were explored regarding the feasibility and appropriateness of introducing verbal autopsies into the current practice.

Data collection procedures

The interview process was conducted by the main researcher. The interviewer contacted each potential participant by phone to introduce the study and arrange an in-person interview, which could be completed in one or multiple sessions, based on the respondent’s preference. Informed consent was obtained for all participants at the first in-person meeting prior to any data collection. All interviews were conducted in the Kurdish language except one, which was done in Arabic, and all interviews were tape recorded in full. Audio recordings were transcribed and translated into English. The translation was subsequently reviewed and verified by another native Kurdish speaker who was fluent in English.

Analysis

Qualitative data analysis was conducted in two phases. The initial phase was reading the English transcriptions thoroughly and extracting key response points from each participant, which produced a table of responses for each question at each stakeholder level. The researchers then consolidated each table by combining responses that had the same meaning and listing the number of respondents who gave each response, producing a summary table of responses that was sorted by frequency for each question at each stakeholder level.

The second phase of analysis involved the summary tables and coded responses. Each set of relevant responses was combined across all the question tables, resulting in three consolidated lists (strengths, gaps, and suggestions) per stakeholder level. Within each list, the responses were then sorted and grouped according to emergent themes. Responses that appeared to address multiple themes were placed within both themes.

Results

The findings of this study are segregated into three main categories: strengths, gaps, and suggestions for
improving the current death registration practices. The most frequently mentioned strength was easy practice and easy for people to get certificate. Based on the perceptions of all the MoH respondents, the current practice can be done easily by staff, but none of the MLI stakeholders saw it as simple. Regarding the stakeholders’ perception of how people who don’t work with death registrations see the death registration system, 87% of all stakeholders indicated that people see it as easy since “they can get death certificate easily.” Half of the MLI respondents consider it a good practice, but neither the MoH nor the DoH respondents agreed with that assessment.

Only 40% of the overall stakeholders (100% of the MoH respondents) agreed that the existence of the laws and regulations related to death registration is strength of the current practices. Having a “standard death certificate” was raised as a strength point by the stakeholders. Follow-up and monitoring of the process of death registration is vigorous at the MLI (100%), whereas none of the other stakeholders considered this a serious task. Regarding training on the International Statistical Classification of Diseases and Related Health Problems (ICD) codes and employing them, MoH stakeholders reported that some staff had been trained, but they hadn’t confirmed if they were employing it: “some staff from statistic departments of some PHCs have being trained on ICD-10 simplified codes... and it seems that they are using them.” However, the MLI stakeholders indicated that they were not using the ICD – 10 and were not trained on the system. Although the MoH stakeholders stated that the practice is simple and good, the accuracy of death registration process was only confirmed by the MLI; both the MoH and DoH stakeholders indicated that the practice is not accurate.

The gaps that were mentioned by the stakeholders were: 73% of all stakeholders raised concerns about the incompleteness of the data; this was markedly higher among the MoH and DoH stakeholders than those at the MLI. Staff ignorance was reported as a prominent cause of the current gap across all stakeholders. Overall, 53% of the participants (with 100% MoH) shared concerns about accuracy and reported that the death registration is not accurate in most of the locations. MLI stakeholders recognized the shortage of health system as a gap, whereas the MoH stakeholders didn’t mention this. Only 40% of the stakeholders identified problems related to the ICD system as a gap. Other gaps identified across stakeholders were no checking of data, missing cases, no use of data, no standardized process of compiling data, and problems with storing data.

Discussion

Many factors affect the completeness and quality of death registration. Staff experience, familiarity with the deceased person, time allocation for the death registration process, and the perception of the importance of death certificate are among the main factors that affect the completeness and accuracy of the death registration. The United Nations Statistics Division highlighted some activities, among many, that proved useful for countries to improve their death registration systems; these included engaging political people in improving civil registration, periodic training of staff, continuous performance monitoring, and maximal use of information technologies related to the systems.

The present study found that there are rules and regulations in the Kurdistan Region for dealing with death registration, but they are not practiced. This is similar to what was found in some Pacific island countries as there is legislation regarding death registration and the weakness is that there is inadequate implementation of such legislation. On the other end, Mathers et al. found that many countries do not appreciate the value of data for public health development and hence they don’t have sufficient procedures and regulations to ensure that death registration processes are done correctly.

One of the similarities among Kurdistan and Pacific island countries is the compilation of death data by various health settings and the usage of standard international medical death certificates, which was identified as a strength factor. There are also similarities in their weaknesses, such as lack of cause of death data for deaths outside health facilities, limited statistical analysis of the registered data, and insufficient quality assessment and control.

The practice of completing a death certificate in Kurdistan is that senior doctors are responsible for assigning the cause of death to their patients, even if they are not at the hospital when their patients die. This is recommended by Bobbie et al. as of the familiarity
of physicians with their patients. They also addressed inexperience, fatigue, time factors, and the perceived lack of importance of the death certificate as factors that affect the accuracy and completeness of death registration, which were similar to what the stakeholders highlighted in the present study.

The suggestions from the stakeholders were similar to those raised by Rao et al. for African countries. These included addressing training needs, quality control, and examining the feasibility of innovative methods, such as verbal autopsy. Including training on how to accurately identify the cause of death in continuing professional development and in medical teaching curricula as well as the training of administrative personnel were previously highlighted as essential strategies in death registration system: these were also emphasized by the stakeholders in this study. A study conducted in Spain by Villar et al. revealed that an educational session can raise the accuracy rate of mortality data by more than three-fold. The stakeholders highlighted the importance of training on ICD codes and this was also raised by Rao et al.

The perspectives of stakeholders related to hiring more staff and conducting training on new systems for improving the completeness and efficiency of death registration in the present study coincides with similar studies conducted in Jordan and Vietnam. Those studies include suggestions like increasing the number of staff and training physicians and other staff who deal with death registration. The importance of training was raised by others as skilled personnel have a critical role in death registration and training provides the knowledge and skills needed for efficient work and hence a better understanding of what is expected and fewer errors.

Conclusion

This study pointed out the stakeholders’ perspectives regarding death registration in the Kurdistan Region of Iraq. They highlighted the existence of rules and regulations and using the standard death certificate format as strength points, among some others, for the current practices. They also mentioned the gaps of the current death registration, including incomplete and inaccurate data, no systematic process of compiling data, no use of data, and staff ignorance. The main suggestions from stakeholders for improving the death registration were emphasizing and implementing the existing rules and regulations, conducting capacity building for doctors and those who deal with death registration, instituting systematic processes for data management, and follow-up and monitoring.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under Department of Community Medicine, College of Medicine, Hawler Medical University, Erbil – Iraq and all experiments were carried out in accordance with approved guidelines.

References


The Effect of Ultrasonic Activation of Two Different Sealers on the Fracture Resistance of Obturated Root Canals

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Abstract

The aim of the current study is to evaluate the effect of ultrasonic activation of two sealers: EndoSequence BC and AH Plus on the fracture resistance of obturated roots.

Materials and Method: Forty eight mandibular first premolars were decoronated to a length of 13 mm from the apex. The roots were divided into two groups (24 samples) to be obturated with gutta percha using either EndoSequence BC(Group I) or AH Plus (Group II) sealers. Each group was further subdivided into two subgroups (n=12). Subgroups IA and IIA were obturated without ultrasonic activation of the sealers. Subgroups IB and IIB were obturated with ultrasonic activation of the sealers. The activation of the sealers was performed using size 15 ultrasonic K-file for a total of 20 seconds 2 mm short of the working length. The samples were subjected to fracture resistance test using a universal testing machine at a across head speed of 0.5 mm/min. The data were analyzed using One way ANOVA and Tukey tests at a significant level of 0.05.

Results: The fracture resistance mean values of the EndoSequence BC subgroups with and without activation were significantly higher than those of AH Plus sealer (P<0.05).

Conclusion: The ultrasonic activation of both sealers did not enhance the fracture resistance of obturated roots.

Keywords: Bioceramic, Epoxy resin based sealer, Ultrasonic.

Introduction

During endodontic therapy, the tooth structure can be compromised increasing the possibility of root fracture due to: removal of supporting tooth structure by access opening and instrumentation, pressure during obturation and dehydration of dentin.¹² The standard obturation material that has been used for years in root canal therapy is gutta percha. However, one of the disadvantages of gutta percha as a filling material is that it does not bond or adhere to the dentinal walls of the root canal resulting in an incomplete sealing of the canal space. It has been shown that the use of sealers with an ability to bond to the root canal dentin will strengthen the remaining tooth structure, thus increasing the root resistance to fracture.³⁴ Nowadays, numerous sealers have been introduced into the market that are supposed to adhere to the root canal dentin such as: methacrylate resin-based sealers, epoxy resin-based sealers, mineral trioxide aggregate (MTA) based sealer and calcium silicate-based sealer. The adhesion and the mechanical interlocking of the sealer can strengthen the remaining tooth structure.⁴ Bolles et al., in 2013⁵ and Macedo et al., in 2014⁶ stated that the use of ultrasonic activation to the endodontic sealers during obturation can increase sealer pressure against the root canal walls allowing better penetration into accessory canals, isthmus, and dentinal tubules, with formation of a greater number, density and extension of tags.⁷⁸ Thus, increasing the adhesion and mechanical interlocking of the filling material.⁹,¹⁰

Endosequence BC sealer is a calcium silicate-based endodontic sealer, with a self-adhesive nature. It has nano-particles permit it to flow readily into the canal’s irregularities and dentinal tubules. In addition, no shrinkage occurs on setting, resulting in a gap-free
interface between the gutta-percha, sealer and dentin.\textsuperscript{11} AH Plus sealer, on the other hand, is an epoxy resin-based sealer characterized by very good mechanical properties, easy handling, potential for better wettability of the dentin and gutta-percha surfaces and self-adhesive property.\textsuperscript{12}

Topcuoglu et al., in 2013\textsuperscript{13} and Yendrembam et al., in 2019\textsuperscript{14} evaluated the fracture resistance of teeth filled with AH Plus and Endosequence bioceramic root canal sealers in vitro. They concluded that both sealers were capable of increasing the force to fracture in single-rooted endodontically treated premolars. Guimarães et al., in 2014\textsuperscript{7}; Wiesse et al., in 2018\textsuperscript{8} and Sachin et al., 2018\textsuperscript{15} evaluated the influence of ultrasonic vibrations on the penetration depth of both sealers using confocal microscopy. They concluded that the ultrasonic activation was linked with higher bond strength values, greater intratubular penetration and better interfacial adaptation to root dentin and less existence of gaps and voids than no activation technique. However, the effect of ultrasonic activation to Endosequence BC and AH Plus sealers on fracture resistance of obturated roots has not been cleared yet. Therefore, this study was conducted to evaluate the effect of ultrasonic activation of Endosequence BC and AH Plus sealers in the assessment of fracture resistance of endodontically treated roots.

**Materials and Methods**

Freshly extracted forty eight single rooted mandibular first premolars were collected after extraction for orthodontic reason from patients with age ranged from (20-30) years old.\textsuperscript{16} The teeth were cleaned from calculus and tissues manually using cumine.\textsuperscript{17} Then, the teeth were stored at room temperature in 0.1 % thymol solution for 48 hs to prevent dehydration and bacterial growth. The teeth were decoronated at a length of 13 mm from the anatomical apex for standardization and to achieve a stable reference during working length determination and instrumentation. The working length was established to be 1 placing a K-file size 10 (Dentsply, Switzerland) into the canal until observing it at the apical foramen.\textsuperscript{13}

For root canal instrumentation, the canals were first irrigated with 1 ml of 2.5 % NaOCl for 1 minute. Then, K-file size 15 was introduced to the full working length to obtain a reproducible glide path. The canals were instrumented using ProTaper Next system (Dentsply Maillefer Endodontics, Switzerland) by the following sequence: X1(17/0.04), X2(25/0.06), X3 (30/0.07) and X4 (40/0.06). For standardization, each canal was irrigated with a total amount of 5 ml of 2.5% NaOCl. Then, a 3 ml of EDTA 17% was applied for 1 minute, then a final irrigation was applied with 10 ml of distilled water.\textsuperscript{13,19} Each canal was dried with two paper points size 40/0.06.\textsuperscript{20}

The roots were randomly divided into two groups (n=24) according to the types of root canal sealers: EndoSequence BC Sealer (Group I) and AH Plus sealer (Group II). Each group was subdivided into two subgroups (n=12): Group IA and Group II A were obturated without sealer activation and Group I B and Group II B were obturated with sealer activation.

For Group IA, a master guttapercha cone X4 (40/0.06) (Dentsply Maillefer) was inserted into the canal to full working length (12 mm) with a good tug-back. EndoSequence BC Sealer was introduced into the root canal by its intracanal tip. The tip was placed into the coronal one third of the canal and the sealer was released while slowly withdrawing the tip from the canal.\textsuperscript{13} Then, the master cone was reinserted and the excess of guttapercha was cut off with a guttapercha cutter at the level of canal orifice.\textsuperscript{20}

For Group IB, the samples were treated as in Group IA except that after the application of the sealer, activation of the sealer was performed using an ultrasonic K-file size 15 in a piezoelectric ultrasonic handpiece (SatelecActeon) at 6 mode power.\textsuperscript{15} Then, the file was activated 2 mm short of the working length, with 2-3 mm back and forth movements in a bucco-lingual and in a mesio-distal direction of the root canal for a total of 20 seconds.\textsuperscript{15}

For Group IIA, the roots were obturated in a similar way using AH plus sealer which was manipulated according to the manufacturer’s instructions. The two pastes were mixed to get a homogenous creamy consistency and the master cone was coated with the sealer and slowly inserted into the canal until the working length was reached.\textsuperscript{14,22} According to the manufacturer instructions, the canal walls were coated with AH Plus sealer through a simultaneously rotating movement of
the master cone in a counter clockwise direction four times per a canal. The excess of gutta-percha was cut off with a gutta-percha cutter at the level of canal orifice.20

For Group IIB, the samples were obturated with ultrasonic activation of AH Plus sealer which was performed in a similar way as in Group IB using the ultrasonic handpiece.

After obturation of the roots of each group, the samples were radiographed to ensure the quality of obturation.13 The samples were stored in an incubator for 1 week at 37°C and 100% relative humidity to permit fully set of the sealers.20 All the procedure was performed by one operator for standardization.

In order to assess the fracture resistance test, the roots were fixed in a cylindrical acrylic block (20 mm height and 20 mm diameter) using a cold-cured acrylic resin to simulate the alveolar bone. While a periodontal ligament simulation was performed by coating the root surface with a thin layer of light-body addition silicon. Then, the roots were embedded into the acrylic resin exposing 2 mm of the coronal parts of the roots.14,20 Fracture resistance test was performed for all roots using an Instron testing machine. A spreader-like metal tip (0.8 mm in diameter) was positioned at the center of each canal orifice and a vertical force was exerted at a across head speed of 0.5 mm/min until fracture.20 The fracture force was recorded in Newton.20

All of the analyses were performed using SPSS 21 software (IBM-SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and post hoc Tukey tests were used to determine the significance difference between the subgroups at P<0.05.

Results

The results of the descriptive statistics are shown in Figure 1. One way ANOVA test showed that there was a significant difference between EndoSequence BC sealer and AH Plus sealer with and without activation (P=0.000). However, the results of Tukey’s test for multiple comparisons between the groups (Table 1) showed non-significant differences neither between the subgroups of EndoSequence BC Sealer (Group IA & Group IB), nor between the subgroups of AH Plus sealer (Group IIA &Group IIB) as P > 0.05.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Difference</th>
<th>S.E</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group IA EndoSequence BC Sealer (Without activation)</td>
<td>Group IB</td>
<td>.083</td>
<td>5.283</td>
</tr>
<tr>
<td></td>
<td>Group IIA</td>
<td>45.083</td>
<td>5.283</td>
</tr>
<tr>
<td></td>
<td>Group IIB</td>
<td>45.250</td>
<td>5.283</td>
</tr>
<tr>
<td>Group IB EndoSequence BC Sealer (With activation)</td>
<td>Group IA</td>
<td>-.083</td>
<td>5.283</td>
</tr>
<tr>
<td></td>
<td>Group IIA</td>
<td>45.000</td>
<td>5.283</td>
</tr>
<tr>
<td></td>
<td>Group IIB</td>
<td>45.167</td>
<td>5.283</td>
</tr>
</tbody>
</table>
Table 1. Tukey test multiple comparisons for the fracture mean values for all subgroups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Fracture Resistance (N)</th>
<th>Group</th>
<th>Mean Fracture Resistance (N)</th>
<th>Group</th>
<th>Mean Fracture Resistance (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group IIA</td>
<td>AH Plus Sealer (Without activation)</td>
<td>Group IA</td>
<td>-45.083</td>
<td>5.283</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>Group IIA</td>
<td>AH Plus Sealer (Without activation)</td>
<td>Group IB</td>
<td>-45.000</td>
<td>5.283</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>Group IIA</td>
<td>AH Plus Sealer (Without activation)</td>
<td>Group IIB</td>
<td>.167</td>
<td>5.283</td>
<td>1.000 (NS)</td>
</tr>
<tr>
<td>Group IIB</td>
<td>AH Plus Sealer (With activation)</td>
<td>Group IA</td>
<td>-45.250</td>
<td>5.283</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>Group IIB</td>
<td>AH Plus Sealer (With activation)</td>
<td>Group IB</td>
<td>-45.167</td>
<td>5.283</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>Group IIB</td>
<td>AH Plus Sealer (With activation)</td>
<td>Group IIA</td>
<td>.167</td>
<td>5.283</td>
<td>1.000 (NS)</td>
</tr>
</tbody>
</table>

Figure 1. Bar chart graph representing the mean fracture resistance (N) of all subgroups.

**Discussion**

It has been reported that during root canal treatment, there will be a reduction in the fracture resistance of the root which consequently leads to tooth extraction.\(^{23,24}\) Hence, one of the essential features of any root canal filling material is their capability to reinforce and significantly strengthen the endodontically treated roots. Recently, researchers illustrated that the adhesion and the mechanical interlocking of the sealers can strengthen the remaining tooth structure, increasing its resistance.
to fracture.4

In this study, the effect of ultrasonic activation of two dentin bonding capable sealers (EndoSequence BC sealer and AH Plus sealer) on the fracture resistance of obturated root canals was evaluated. The results showed that the mean values of fracture resistance of both subgroups of EndoSequence BC sealer with and without activation were significantly higher than AH Plus sealer subgroups. Significantly higher fracture resistance for bioceramic sealers vs AH Plus sealer were also reported by recent studies using Totall fill biocermic sealer.25,26 Two other studies reported similar results using Endosquence bioceramic sealer.14,27 Reasons for such a result were related to the deeper penetration of the nanoparticles of EndoSequence BC sealer into the dentinal tubules and canal irregularities. EndoSequence BC sealer has a nanoparticle size of an average of 0.2 µm, which could increase the distribution of particles into dentinal tubules particularly within the apical root area.28 AH Plus sealer, on the other hand, and according to the manufacturer, contains calcium tungstate particles with an average size of 8 µm and zirconium oxide particles with a 1.5 µm size. The larger particles could not enter easily into the small tubules and the sealer tags would be weaker than those of bioceramic sealer.20 Additionally, Yendrembam et al., in 201914 showed that EndoSequence BC sealer has a higher adhesiveness to the root dentin than AH Plus sealer. EndoSequence BC sealer create a chemical and micromechanical bonding with dentin by means of the production of hydroxyapatite during setting, in contrast to a limited adhesion of AH Plus sealer to dentin from a mild covalent bond between its open epoxide ring and the amino group in the collagen fiber.29,30 Oltra et al., in 201731 reported that EndoSequence BC sealer had a significantly more residual filling material than AH Plus sealer after removal of the sealers indicating higher interlocking of EndoSequence BC sealer. Another possible reason could be related to polymerization shrinkage of AH Plus sealer associated with the use of large amounts of sealer in the canal when used with a single cone technique.32,33 In contrast, the EndoSequence BC sealer is a hydrophilic in nature and has no shrinkage11,34 and it expands 0.2% on setting producing a self-seal. This expansion together with the chemical and micromechanical bonding could improve the bonding of the root canal sealer to the canal walls.35,36

Topçuoğlu et al., in 201313 reported a higher, however, not significantly different fracture resistance mean value of EndoSequence BC sealer compared with AH Plus sealer. Such a difference could be related to the differences in methodologies. In this study, a spreader-like of 0.8 mm diameter metal tip with a vertical force in 0° angle, resulting in splitting stress exerted along the long axis of the root. Additionally, the roots had only 2 mm exposed above the acrylic block. This resulted in smaller stresses because of decreased bending movements and maximum stresses situated more cervically.20,37

It has been reported by many studies that the activation of endodontic sealers might improve root canal sealing, predominantly in areas of difficult access for example lateral and accessory canals, isthmus, recesses, and apical deltas.7,8 Numerous studies illustrated that the use of ultrasonic activation of these sealers cause greater dentinal sealer penetration and less presence of gaps, and increasing the adherence to the root dentin.7,8,15,21 However, the result of this study showed that the ultrasonic activation to both sealers produced no significant effect on the fracture resistance of obturated roots. This could be explained primarily as higher penetration can be different in different levels of the root. Better penetration due to sealer activation can be seen for both sealers in the apical more constricted areas than the coronal area.7,8 Therefore, further investigations and studies are still needed to verify the effect of sealer activations on its adhesion to root dentin at different root levels.

Conclusions

Under the circumstances of this study, the following conclusions are withdrawn:

1. EndoSequence BC sealer group showed a significantly higher fracture resistance than AH Plus sealer group with and without activation.

2. Ultrasonic activation of both EndoSequence BC and AH Plus sealers produced no significant influence on the fracture resistance of obturated roots.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.
**Ethical Clearance:** All experimental protocols were approved under the College of Dentistry and all experiments were carried out in accordance with approved guidelines.

**References**

21. Prasad P K, Sankhala A, Tiwari A, Parakh S, Madan GR, Singh A. Influence of ultrasonics on the penetration depth of AH plus, acroseal, and...


Hepato-nephroprotective Role of *Lepidium sativum* against Oxidative Stress Induced by Dexamethasone in Rats

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**Abstract**

The current research was prepared to explore the hepato-nephroprotective effect of *Lepidium sativum* seed aquatic extract against oxidative stress stimulated by dexamethasone in rats. Animals were classified as follows; group one was used as control while groups 2, 3 and 4 were treated orally with *Lepidium sativum* seed extract (LSSE; 20 mg/kg, daily), interperitoneally with dexamethasone (DEX; 1 mg/kg BW) and LSSE plus DEX for 14 days, respectively. Administration of DEX elevated thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H$_2$O$_2$), and kidney and liver function biomarkers level, and lactate dehydrogenase activity. While enzymatic (SOD, CAT, GPx, GR, GST) and non-enzymatic (GSH) antioxidants, protein content, and alkaline phosphatase activity were significantly decreased. Otherwise, rats supplemented with LSSE singly declined lipid peroxidation and improved most of the studied parameters. Moreover, rats pretreated with LSSE then received DEX showed significant alleviation in lipid peroxidation, antioxidant status and biochemical indices with respect to DEX treated group. Conclusively, LSSE has beneficial protective effects and has the capability to counteract the harmful influence of DEX. So, *Lepidium sativum* might represent a novel approach in the therapy of dexamethasone because of its antioxidant properties.

**Keywords:** Dexamethasone; *Lepidium sativum*; oxidative stress; antioxidant enzymes; liver and kidney dysfunction.

**Introduction**

Glucocorticoids (GCs), the primary stress hormones secreted by adrenal glands, are essential for life. Several synthetic glucocorticoids such as dexamethasone, prednisone, and betamethasone are more potent than natural cortisol. They are involved in the treatment of disturbances induced by an overactive immune system as allergies, asthma, autoimmune diseases and sepsis. Additionally, GCs are involved in several diseases including obesity, metabolic syndrome, hypertension, and depression. GCs have several side effects including the activation of the mineralocorticoid receptors leading to the growth of renal injury. However, dexamethasone that given in vivo can also induce albuminuria and promote also renal injury. Furthermore, high GCs concentrations result in overproduction of reactive oxygen species (ROS) leading to alteration in mitochondrial permeability, cellular energy and calcium levels followed by apoptosis. The over production of ROS in dexamethasone therapy is predominantly due to increased NADPH oxidase activity. Previous study showed that dexamethasone treatment prompted gluconeogenesis, increased insulin resistance in addition to lipid disturbances. Reactive oxygen species are ordinarily created in a regulated manner in human and animals. Nevertheless, xenobiotics can provoke oxidative stress and consequently hurt the cellular macromolecules. Essentially, the cellular defense mechanism versus ROS toxicity involved non-enzymatic (glutathione (GSH), vitamin E, ascorbic acid, β-carotene, and uric acid) and enzymatic antioxidants (superoxide dismutase, catalase, selenium-dependent glutathione peroxidase, selenium-independent glutathione peroxidase, alkyl hydroperoxide reductase, glutathione S-transferase and glutathione reductase).

Great interest is directed to many plants because of their antioxidant and anti-inflammatory potential, among them *Lepidium sativum* Linn which belongs to...
Cruciferae family. *Lepidium sativum* is known as “El-Rshad” is an edible herb with aroma flavor. In traditional medicine, different parts of this plant have been adopted for several diseases therapy (jaundice, liver problems, spleen diseases, gastrointestinal disorders, menstrual problems, arthritis, asthma, headache, nasal polyps, breast cancer, and other inflammatory conditions)\(^8,9\). *Lepidium sativum* seeds essential oils contains fatty oils, protein, carbohydrate, vitamins, fatty acids, flavonoids, and isothiocynates glycoside\(^10\). Interestingly, experimental evidences showed that *Lepidium sativum* has multiple useful properties as antihypertensive, diuretic\(^38\), anti-asthmatic\(^37\), hypoglycaemic, antioxidant\(^10\) and anti-inflammatory\(^39\). Therefore, the existent study was prepared to evaluate the hepato-renal protective effect of *Lepidium sativum* seed aqueous extract against oxidative stress and biochemical perturbations induced by dexamethasone in rats.

### Materials and Methods

#### Materials

*Lepidium sativum* seeds (LSS) were collected from local market of Baghdad, Iraq and stored in air tight containers. Dexamethasone phosphate (DEX ≥ 98%), was bought from Sigma Chemical Company. All other chemicals were of analytical grade.

**Lepidium sativum seed extract preparation**

*Lepidium sativum* seed aqueous extract was prepared by boiling 1 g of dry seeds powder in 100 ml of distilled water for 10 min and left for 15 min to soak then chilled and filtered. The obtained filtrate was lyophilized and the required dose was prepared and reconstituted in 10 ml of distilled water per kilogram bodyweight prior oral administration directly.

**Experimental design**

Twenty-eight male Wister rats (150–170 g) were bought from the Faculty of Medicine, Alexandria University, Alexandria, Egypt. The protocol was approved by the local University Committee in conformity with the ethics and guidelines of the National Institutes of Health. Rats were distributed in cages seven per each and kept on commercial diet and water *ad libitum* and acclimated (temperature, 21°C; photoperiod, 7 a.m. to 7 p.m.) for two weeks. Animals were classified into four groups: group 1 used as the control, while group 2 was treated orally with *Lepidium sativum* seed aqueous extract (LSSE; 20 mg/kg), group 3 was injected interperitoneally with dexamethasone (DEX; 1 mg/Kg) dissolved in saline and the fourth group received LSSE one hour before DEX treatment, respectively. *Lepidium sativum* and dexamethasone were administered daily for 14 days according to\(^19\). At the experiment termination, rats were anesthetized using isoflurane, and then killed via cervical dislocation and livers and kidneys were immediately removed.

#### Blood and tissue samples

Blood samples were assembled for serum preparation and permitted to stand for 30 min for blood clotting at 25°C then centrifuged at 3000 ×g for 15 min. Serum of each sample was taken and stored at −20°C till utilized in the determination of biochemical parameters. Livers and kidneys were taken away and homogenized in ice-cold 0.01 mol/l sodium-potassium phosphate with 1.15% KCl buffer (pH 7.4) and centrifuged at 10,000 g (4°C) for 20 min then the supernatants were taken and utilized for the determination of different assays.

**Determination of TBARS, H\(_2\)O\(_2\) and glutathione content**

Thiobarbituric acid-reactive substances (TBARS), hydrogen peroxide (H\(_2\)O\(_2\)), and reduced glutathione (GSH) content were measured in liver and kidney homogenates using the methods of Ohkawa et al. (1979), Velikova et al. (2000), and Ellman (1959), respectively.

**Determination of antioxidant enzyme activities**

The activities of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione S-transferase (GST; EC 2.5.1.18) were assessed by the methods of Misra and Fridovich (1972), Aebi (1984) and Habig et al. (1974), respectively. While the activities of glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC 1.6.4.2) were evaluated according to Hafeman et al. (1974).

**Determination of biochemical parameters**

Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed using kits from SENTINEL CH.
Lactate dehydrogenase (LDH; EC 1.1.1.27) and alkaline phosphatase (ALP; EC 3.1.3.1) activities, protein content, and total bilirubin were estimated by the methods of Cabaud and Wroblewski (1958), Principato et al. (1985), Bradford (1976) and Walters and Gerade (1970), respectively. Urea and creatinine concentrations were measured according to Patton and Crouch (1977) and Henry et al., (1974), respectively.

**Statistical analysis**

Data from different groups were represented as means ± standard errors (SEM) then analyzed utilizing SPSS software (version 22, IBM Co., Armonk, NY). Comparison between groups was done through one-way ANOVA followed by Tukey’s post-hoc test. P value ≤ 0.05 was approved to be significant.

**Results**

**General health**

None of the dexamethasone treated rats showed signs of morbidity or mortality during the study.

**Lipid peroxidation and reduced glutathione**

The present results revealed significant (P< 0.05) increase in TBARS and H$_2$O$_2$ levels, the indicators of lipid peroxidation, in liver and kidney homogenate after DEX treatment versus control while rats pretreated with LSSE then treated with DEX presented a significant reduction in TBARS and H$_2$O$_2$ levels as compared to DEX- treated rats. On the other hand, GSH content was significantly decreased in DEX -treated rats. While rats treated with both LSSE and DEX, induction in GSH content was observed as compared with DEX- treated rats. Supplementation with LSSE alone reduced the concentrations of TBARS and H$_2$O$_2$ and induced GSH content in liver (Table 1) and kidney (Figure 1) homogenates.

**Antioxidant enzymes**

A significant reduction (P<0.05) in SOD, CAT, GPx, GR, and GST activities was observed in liver (Table 2) and kidney (Figure 2) homogenates of DEX- treated rats. Furthermore, rats taken LSSE + DEX showed significant alleviation in the activities of antioxidant enzymes as compared to DEX - treated ones (P<0.05). Moreover, LSSE supplementation alone improved antioxidant enzyme activities significantly versus the control group.

**Liver and kidney function biomarkers**

Data showed that AST, ALT, and ALP activities and protein content decreased while LDH activity as well as serum urea, creatinine and total bilirubin increased significantly (P < 0.05) in rats received DEX as compared to control. Moreover, a significant modulation in enzyme activities and protein content in rats received LSSE then treated with DEX versus DEX group was observed. LSSE supplementation alone had insignificant alteration on the enzymes and protein contents in (Table 3 and 4).

**Table 1.** Effect of *Lipidium sativum* seed extract (LSSE), dexamethasone (DEX) and their combination on the level of thiobarbituric acid reactive substances, hydrogen peroxide and reduced glutathione content in rat liver

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont.</td>
</tr>
<tr>
<td>TBARS (nmol/g tissue)</td>
<td>36.74±0.951c</td>
</tr>
<tr>
<td>H$_2$O$_2$ (μmol/g tissue)</td>
<td>64.56±2.05c</td>
</tr>
<tr>
<td>GSH (mmol/mg protein)</td>
<td>2.19±0.071b</td>
</tr>
</tbody>
</table>
Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, \( p < 0.05 \). Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.

### Table 2. Effect of *Lipidium sativum* seed extract (LSSE), dexamethasone (DEX) and their combination on the activities of antioxidant enzymes in rat liver

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Cont.</th>
<th>LSSE</th>
<th>DEX</th>
<th>LSSE + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td></td>
<td>70.59±1.89b</td>
<td>84.48±2.46a</td>
<td>36.99±1.19d</td>
<td>54.72±1.45c</td>
</tr>
<tr>
<td>CAT (µmol/hr/mg protein)</td>
<td></td>
<td>41.57±1.38b</td>
<td>49.29±1.15a</td>
<td>22.73±0.966d</td>
<td>32.71±1.08c</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td></td>
<td>1.19±0.040b</td>
<td>1.42±0.052a</td>
<td>0.688±0.026d</td>
<td>0.954±0.026c</td>
</tr>
<tr>
<td>GR (U/mg protein)</td>
<td></td>
<td>1.31±0.041b</td>
<td>1.56±0.059a</td>
<td>0.717±0.023d</td>
<td>1.038±0.027c</td>
</tr>
<tr>
<td>GST (µmol/hr/mg protein)</td>
<td></td>
<td>0.984±0.033b</td>
<td>1.17±0.041a</td>
<td>0.565±0.021d</td>
<td>0.802±0.020c</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, \( p < 0.05 \). Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.

### Table 3. Effect of *Lipidium sativum* seed extract (LSSE), dexamethasone (DEX) and their combination on the enzyme activities and protein content in rat liver

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Cont.</th>
<th>LSSE</th>
<th>DEX</th>
<th>LSSE + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver AST (U/mg protein)</td>
<td></td>
<td>88.94±2.45a</td>
<td>92.28±2.04a</td>
<td>52.30±1.68c</td>
<td>73.20±1.89b</td>
</tr>
<tr>
<td>ALT (U/mg protein)</td>
<td></td>
<td>119±4.09a</td>
<td>115±4.12a</td>
<td>71.49±2.59c</td>
<td>95.22±1.32b</td>
</tr>
<tr>
<td>LDH (U/mg protein)</td>
<td></td>
<td>659±20.62c</td>
<td>630±20.30c</td>
<td>919±17.79a</td>
<td>793±20.35b</td>
</tr>
<tr>
<td>ALP (U/mg protein)</td>
<td></td>
<td>360±9.07a</td>
<td>381±10.17a</td>
<td>216±7.00c</td>
<td>288±5.94b</td>
</tr>
<tr>
<td>protein (mg/g tissue)</td>
<td></td>
<td>171±4.81a</td>
<td>176±3.28a</td>
<td>103±5.07c</td>
<td>146±5.94b</td>
</tr>
<tr>
<td>Serum Total bilirubin (mg/dl)</td>
<td></td>
<td>0.727±0.015c</td>
<td>0.703±0.021c</td>
<td>0.966±0.036a</td>
<td>0.817±0.82b</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n=7 for each treatment group. Mean values within a row not sharing common superscript letters were significantly different, \( p < 0.05 \). Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.
Figure 1. Effect of *Lipidium sativum* seed extract (LSSE), dexamethasone (DEX) and their combination on the level of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H$_2$O$_2$) and reduced glutathione (GST) content in rat kidney. Values are expressed as means ± SE; n=7 for each treatment group. Mean values with different letters were significantly different, *p*< 0.05.
Figure 2. Effect of *Lipidium sativum* seed extract (LSSE), dexamethasone (DEX) and their combination on the antioxidant enzymes activity in rat kidney. Values are expressed as means ± SE; n=7 for each treatment group. Mean values with different letters were significantly different, *p*< 0.05.
Table 4. Effect of *Lipidium sativum* seed extract (LSSE), dexamethasone (DEX) and their combination on the concentration of urea and creatinine, enzyme activities and protein content in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont.</td>
</tr>
<tr>
<td>Serum Urea (mg/dl)</td>
<td>38.49±0.949c</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.699±0.025c</td>
</tr>
<tr>
<td>Kidney LDH (U/mg protein)</td>
<td>617±22.82c</td>
</tr>
<tr>
<td>ALP (U/mg protein)</td>
<td>183±6.27a</td>
</tr>
<tr>
<td>Protein (mg/g tissue)</td>
<td>60.04±2.05a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n=7 for each treatment group. abcdMean values within a row not sharing common superscript letters were significantly different, p<0.05. Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.

**Discussion**

In the current investigation, the protective role of LSSE against DEX - induced oxidative injury and biochemical perturbations was studied. To our knowledge there are no findings have been pointed out the efficiency of LSSE as natural products for overcoming DEX harmful effect. Dexamethasone is known to disrupt oxidants/antioxidant balance in tissues, leading to various biochemical and physiological dysfunctions. Rats treated with DEX exhibited an imbalanced oxidant/antioxidant status as apparent in TBARS and H$_2$O$_2$ elevation accompanied by depletion in enzymatic (SOD, CAT, GPx, GR, GST) and non-enzymatic antioxidants (GSH) in the liver and kidney homogenates indicating the failure of antioxidant defense system to overcome the flow of ROS. The current results are in consistent with Kiersztan et al. (2017) and Jatwa and Kar (2010) who demonstrate the generation of hydroxyl free radicals and lipid peroxidation in the kidney of rabbits and mice treated with dexamethasone, respectively. Moreover, several authors showed that dexamethasone induced ROS generation in different tissues and cells via the mitochondrial electron transport chain, vascular endothelial xanthine oxidase and NADPH oxidase (Almeida et al., 2011; Feng and Tang, 2014; Assaf et al., 2012). So, this study showed that administration of DEX impaired antioxidant defense system since it has the capability to induce oxidative stress.

Antioxidant enzymes are so important in the preservation of homeostasis for normal cell function in addition they are used as indicators of oxidative stress (Gutteridge, 1995). Superoxide dismutase, as part of the defense system versus oxidative hurt in aerobic organisms, catalyzes superoxide anion (O$_2^-$) to O$_2$ and H$_2$O$_2$, which then is reduced to H$_2$O by H$_2$O$_2$-scavenging enzyme, catalase. The decrease in both SOD and CAT activities might be related to inhibition of enzyme protein synthesis. Glutathione plays a crucial role in cellular protection versus xenobiotics toxicity because of its thiol group (Halliwell and Gutteridge, 2007). Glutathione acts as a reducing non-enzymatic antioxidant and as a substrate for GPx and GST antioxidant enzymes (Cosso et al., 1997). GPx preserves the lipid-cellular membrane from oxidative injury (Kantola et al., 1988) and spurs hydroperoxide reaction with reduced glutathione to form disulphide glutathione (GSSG) (Kalaiselvi et al.,...
While, glutathione S-transferases (GSTs), play a critical role in the detoxification process of xenobiotic to non-toxic products, protecting against electrophiles and oxidative stress (Ghosh et al., 2012). Moreover, inhibition of GST activity in the liver and kidney of DEX-treated rats might repress the elimination of cellular free radicals leading to renal damage displayed by elevation of lipid peroxidation and creatinine (Kiersztan et al., 2017). DEX may affect the synthesis of GSH through the inhibition of glutathione-synthase and glucose 6-phosphate dehydrogenase activities. Additionally, DEX retards the diversion of oxidized glutathione (GSSG) into its reduced form (GSH) via GR inhibition (Yeh et al., 2005). In consistence with the present results, GCs may also indirectly participate in the induction of oxidative stress through inhibiting antioxidant enzymes activity. So, antioxidant enzymes, which prohibit the chain reaction of free radicals, are so important in alleviating DEX hurtful effect in liver and kidney tissues.

The induction in kidney function biomarkers (urea and creatinine) in DEX-treated rats reflected the renal dysfunction. This may be attributed to the metabolic impairment in liver function, as urea is the end-product of protein breakdown. While, high creatinine concentration is related to muscle creatine catabolism that leads to kidney damage (El-Demerdash and Nasr, 2014). Also, DEX may contributes to disturbances in kidney functions, resulting in development of albuminuria which is considered as an early indicator of renal damage or inflammation (Xu et al., 2010).

Xenobiotics are transformed in the liver into less harmful products leading to hepatocytes damage. In the current study, rats received DEX showed significant variations in AST, ALT, ALP and LDH activities as well as total bilirubin and protein levels. These parameters are important biomarkers for hepatocellular damage (Chen et al. 2019) and its alterations pointed out hepatocytes damage that altered the transport function and membrane permeability as well as leakage of enzymes from the cells to the bloodstream indicating hepatotoxicity (Gokcimen et al., 2007; Chen et al. 2019; Albasher et al., 2020). Also, lipid peroxidation has a fundamental role in the disruption of hepatocellular membrane integrity, leading to the leakage of cytoplasmic enzymes and this confirmed the possible mechanism of oxidative stress in liver lesion induced by DEX (Bhaduria, 2012). Lactate dehydrogenase is known as a potent marker in the toxicity assessment of many xenobiotics. The observed induction in LDH activity in DEX-treated rats may be attributed to cellular decay that leads to impairment in carbohydrate and protein metabolism in addition to energy depletion (Sivakumari et al., 1997). Alkaline phosphatase is an important membrane-bound enzyme used as a biomarker for xenobiotics toxicity and critical enzyme in the biological processes. It is responsible for detoxification, metabolism, and biosynthesis of macromolecules that are required for many biological functions and its inhibition in organs could be attributed to tissue necrosis that leads to seepage of the enzyme into the bloodstream (Yarbrough et al. 1982). The elevation in total bilirubin may be related to diminished liver uptake, conjugation, or prolonged bilirubin output from hemolysis (El-Demerdash, 2004). Protein is an essential cellular component susceptible to damage by free radicals and its depression might be because of exaggerated leakage via nephrosis (Chatterjea and Shinde 2002). Additionally, the decrease in protein may be related to disturbance in protein anabolic and catabolic processes.

Our results inferred that LSSE pre-supplementation significantly repaired the liver and kidney biomarkers induced by DEX confirming its guarding potential and its important role in preventing oxidative stress related liver damage due to its anti-inflammatory effect. Protective and therapeutic use of ethanolic LSSE in rat’s renal failure reduced urea and creatinine concentration significantly indicating excess of glomerular filtration rate. Also, administration of aqueous LSSE for 3 weeks exhibited antihypertensive and diuretic activities and suppress free radical attack in rats. So, this study is a successful attempt that introduces *Lepidium sativum* as a wonderful antioxidant for mitigation of DEX-induced oxidative stress in rats due to its antioxidant properties.

**Conclusion**

In conclusion, the present results pointed out that dexamethasone has the potency to cause hepatorenal dysfunction via oxidative injury, alterations in antioxidant defense system, enzyme activities, and biochemical parameters. Furthermore, *Lepidium sativum* administration in combination with dexamethasone...
attenuates its harmful effect by quenching and chelating free radicals. So, *Lepidium sativum* had a powerful antioxidant role in alleviating dexamethasone side effects by potentiating antioxidant defense system status and depressing free radicals’ generation.

**Competing of Interest:** The author reports no conflict of interest.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Misan University and all experiments were carried out in accordance with approved guidelines.

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An Updated Review on Some Neurotoxic Pharmacological Agents Along with their Neurotoxic Mechanisms

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Abstract

This review will provide the vital information about some pharmacological agents which are Neurotoxic. Through this update, Anti-cancer, Anti-bacterial, Analgesic, Psychoactive drug and Anabolic steroid medication are reported briefly regarding their neurotoxic mechanisms. In this review all information regarding neurotoxic drugs is collected from 2020 published work by Web of science, Scopus, PubMed and Google Scholar. It is concluded that all these drugs which are part of our study are neurotoxic. There is need to discover some method to reduce their toxicity and to avoid the chronic use of these medications.

Key Words: Neurotoxic, Pharmacological agents, Anti-cancer, Analgesic, Antibacterial

Introduction

Several types of drugs have side effects on brain, some are minimal, and some are toxic. Toxic effects of drug on brain can cause irreversible brain damage sometimes which are very dangerous for mental health, as any living organism can lose its life due severe neurotoxicity ¹. In routine treatments we don’t know about some drugs which we are taking has negative impact on our mental health as well as on our nervous system ².

Neurotoxicity is a major side effect of many chemotherapeutic drugs used for the treatment of many diseases, including tumors. Toxicity can compromise the quality of life of patients. As per previous reports 84.4% of patients which were affected by lymphoma and treated with chemotherapeutic agents developed a serious neuropathy regarding sensory organs and 43.8% showed polyneuropathy, causing a significantly dangerous for the quality of life ³.

Such effects are usually cause spontaneously fade, as doses are high then chances of neurotoxicity are also high ⁴. If these drugs are used at high therapeutic levels for long time, the plasticity of the neurons is affected badly, and the damage becomes irreversible sometimes ⁵.

Neurotoxicity is one of the main reasons of drug withdrawal, and the biological experimental methods of evaluating neurotoxicity are time taking and arduous. Many Anti-biotic, Analgesic are also showed their neurotoxicity due to their cytotoxic and neurochemical disturbing mechanisms ⁶.

In this updated review, adverse effects of some drugs that cause neurotoxicity are explained individually. These updates are collected from recently published work in 2020. Among these drugs, anti-cancer, anti-bacterial, Analgesic, Psychoactive drug and Anabolic steroid medication are reported briefly. Table 1 is showing summarized report regarding some neurotoxic drugs with their mechanism.

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## Table1: Neurotoxic Drugs with mechanisms

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Neurotoxic Mechanism</th>
<th>Year of Publish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
<td>Anti-cancer</td>
<td>Apoptosis in PC12 is a cell line derived from a pheochromocytoma of the rat adrenal medulla, that have an embryonic origin from the neural crest that has a mixture of neuroplastic cells and eosinophilic cells.</td>
<td>2020</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Anti-cancer</td>
<td>It interact with nuclear DNA and impairing expression of proteins synthesis, involved in mitochondrial functions</td>
<td>2020</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Anti-cancer</td>
<td>It also interreacts with DNA, forms crosslinks in between chains and induces apoptosis. It can cause Central and Peripheral neuropathy</td>
<td>2020</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>Anti-cancer</td>
<td>Inhibition of the DNA repair pathways, generation of DNA adducts in brain cells</td>
<td>2020</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Anti-cancer</td>
<td>Alterations in voltage-gated sodium channel kinetics, Potassium channel blockade, Calcium chelation and sensory axonal nerve damage</td>
<td>2020</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>Anti-cancer</td>
<td>Ifosfamide inhibits the DNA functions and induces cell death.</td>
<td>2020</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Anti-cancer</td>
<td>Interferences with DNA synthesis; inhibits of thymidylate synthase; blocks of thymidine formation</td>
<td>2020</td>
</tr>
<tr>
<td>Novel Methcathinones</td>
<td>Psychoactive</td>
<td>Mitochondrial toxicants whose toxicity is increased by shifting the temperature from 37 to 40.5 °C (Hyperthermia)</td>
<td>2020</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Anti-cancer</td>
<td>Inhibition of the enzyme dihydrofolate reductase; interference with DNA synthesis, DNA repair, cellular replication, protein synthesis, lipids and myelin metabolism. Motor and autonomic neuropathy)</td>
<td>2020</td>
</tr>
<tr>
<td>Cefepime</td>
<td>Anti-Bacterial</td>
<td>Cross the blood brain barrier and cause depressed concentration of consciousness, confusion, aphasia, asterixis, myoclonus, dystonia, seizure in 23.2% population.</td>
<td>2020</td>
</tr>
<tr>
<td>Nandrolone decanoate</td>
<td>Anabolic steroid</td>
<td>Oxidative stress, inflammation, and intrinsic and extrinsic apoptosis in the hippocampus and PFC of rats</td>
<td>2020</td>
</tr>
<tr>
<td>Cannabis</td>
<td>Psychoactive</td>
<td>Apoptosis in the hippocampus and PFC of rats</td>
<td>2020</td>
</tr>
<tr>
<td>Tramadol</td>
<td>Analgesics</td>
<td>It can trigger the microgliosis and astrogliosis along with neuronal death in the prefrontal cortex</td>
<td>2020</td>
</tr>
<tr>
<td>N-Ethylhexedrone and buphedrone</td>
<td>Novel Psychoactive</td>
<td>Neuro-microglia dysfunctionalities</td>
<td>2020</td>
</tr>
</tbody>
</table>
**Bortezomib**

Bortezomib is an anti-tumor agent that inhibits 26S proteasome degrading proteins. While apoptotic transcription activation in response to bortezomib has also been observed, mechanisms regarding influence on gene silencing mediated regulation by non-coding RNAs remain not fully explained. Bortezomib showed the severe neurotoxicity through apoptosis in PC12 cells. It imparts neurotoxicity regardless of cell density. Some studies also showed that, highest cytotoxicity in low cell density, bortezomib more frequently cause major peripheral neuropathy, only few of studies have reported the effective strategy to prevent its side effect.

**Doxorubicin**

Doxorubicin is the most potent anthracycline antibiotics used for treatment of multiple cancer types including breast cancer therapy. But its efficacy is limited by fatal toxicities associated with therapy causing damage to normal tissues and organs. It Interacts with nuclear DNA, altering the pair base sequence and preventing the topoisomerase-II-mediated DNA repair mitochondrial DNA, impairing expression of proteins involved in mitochondrial functions.

**Cisplatin**

Cisplatin is an anti-cancer drug it interreacts with DNA, forms crosslinks between chains and induces apoptosis. Central or Peripheral neuropathy. 50–85% score of sensory and sensorimotor neuropathy. Oxidative stress, generation of DNA adducts, apoptosis, mitochondrial dysfunction are also among its neurotoxic mechanisms. Cisplatin also react with RNA but the ration of modified molecules to the total number of the same molecular species in the cell is much higher in the case of DNA molecules, binding of this agent to the DNA is the main cause of its toxicity.

**Carboplatin**

Carboplatin is widely used agent to treat the various types of cancer. However, a number of severe side effects induced by the nonspecific binding of platinum drugs to normal tissues limit their clinical use. This drug is also involved in Inhibition of the DNA repair pathways, generation of DNA adducts and cause severe neurotoxicity.

**Oxaliplatin**

Oxaliplatin is involved in acute neuropathy upon exposure to chronic peripheral neuropathy, in which sensory axonal nerve abnormal generation of DNA adducts is occurred. Mostly due to Alterations in voltage-gated sodium channel kinetics, Calcium chelation and Potassium channel blockade. Peripheral neurotoxicity is a main toxicity that afflicts up to 90% of patients with colorectal cancer which are taking oxaliplatin containing therapy.

**Ifosfamide**

Ifosfamide is an alkylating agent used in the treatment of various solid tumors, including small cell lung cancer, testicular cancer, cervical cancer, and sarcoma. It is Pro-drug, that after bioactivation, inhibits the DNA functions and cause cell death. Chloroacetaldehyde (metabolite responsible for neurotoxicity) inducing many damages to the mitochondrial respiratory chain depletion of glutathione level in central nervous system and also creating oxidative stress.

**5-Fluorouracil**

This drug also reacts with DNA synthesis and inhibits of thymidylate synthase which blocks the thymidine formation. Seldomly sensorimotor polyneuropathy is observed during treatment with 5-Fluorouracil. Maximum doses and combined use of 5-FU with interferon alpha increases the neurotoxicity. It impairs the urea cycle and permits an accumulation of ammonia a transient stagnation of 5-FU catabolites induces neurotoxicity. This drug can increase the cellular thiamine metabolism, inducing a thiamine deficiency.

**Novel Methcathinones**

These are mitochondrial toxicants whose toxicity is increased by transferring the temperature from 37 to 40.5 °C. It can cause apoptosis and necrosis among brain cells. The activation of proper defense mechanisms like autophagy is necessary to prevent the cell dysfunction and cell death. Mitochondrial toxicity, which is accentuated by hyperthermia, represents an important mechanism of the neural toxicity of these compounds.
Methotrexate

Methotrexate is a folic acid antagonist for the treatment of cancer and rheumatoid arthritis because of its high potency and efficacy. It inhibits the enzyme dihydrofolate reductase which interference with DNA synthesis, DNA repair, protein synthesis, cellular replication, lipids and cause autonomic neuropathy. Aseptic meningitis occurs in 10–50% of patients due to influence of this drug. Disturbances of myelin metabolism, inhibition of glucose metabolism, oxidative stress.

Cefepime

Cefepime is an antibacterial drug belongs to cephalosporins, previously its concentrations were determined in 584 individuals. Among 319 individuals with available through concentrations included, the overall incidence of neurotoxicity was 23.2% (74 of 319 individuals). Maximum cefepime plasma trough concentrations were significantly associated with risk of neurotoxicity. Possible adverse neurological effects based on the occurrence of neurological signs (altered mental status, depressed concentration of consciousness, aphasia, myoclonus, asterixis, confusion, seizure, dystonia).

Nandrolone decanoate with Cannabis

These are Psychoactive drugs. Polydruge use among adolescence is a widespread activity and has enhanced in the last some years. Most nandrolone decanoate abusers combine its use with cannabis. Abuse of both drugs conferred larger neurotoxic effects than either drug alone that were at least partially attributed to inflammation, oxidative stress, and apoptosis in the hippocampus and prefrontal cortex of brain in rats.

Tramadol

Tramadol is a synthetic analogue of codeine that is prescribed for the treatment of moderate pains as an analgesic. It has also some side effects including emotional instability and anxiety. It triggers astrogliosis and microgliosis along with neuronal death in the prefrontal cortex. Behavioral problems and cognitive function impairment are other side effects of tramadol. Previous results indicate that tramadol is responsible for neurodegeneration in the prefrontal cortex through activation of neuroinflammatory response.

Cathinones N-Ethylhexedrone and buphedrone

N-Ethylhexedrone and buphedrone are emerging synthetic cathinones. Small information about their negative effects within central nervous system. These drugs showed in vivo/in vitro neurotoxicity’s but enhanced specific N-Ethylhexedrone induced behavioral abnormalities.

Conclusion

Due to reported neurotoxic mechanisms of all discussed drugs in this review, it is concluded that long term use of these medication can cause brain damaged. There is need to find some methods to overcome the neurotoxic effects of all these drugs.

Source of support: We acknowledge USM (Universiti Sains Malaysia) fellowship scheme support for Muhammad Irfan Bashir.

Conflict of Interest: No any conflict of interest

Ethical Declaration: This is a review article so there is no need to get its ethical approval.

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CT scan Findings of COVID-19 in Patients with Fatty Liver

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Abstract

Purpose: COVID 19) is a respiratory disease caused by a member of the coronaviruses family, named (SARS-CoV-2), discovered in December 2019 in China. This study aims to assess the association of the simple fatty liver disease with the severity of the chest computed tomography (CT) scan findings in COVID 19.

Materials and Methods: It was a cross-sectional study that enrolled 322 patients with positive (RT-PCR) tests and chest CT scan findings. Patients with chronic lung and liver disease, alcoholics, and abnormal liver function tests excluded from the study. The liver CT scan density assessed, accordingly the patients divided into fatty-liver and non-fatty liver groups. The severity of lung CT changes compared between them, using Chi-square test.

Conclusion: COVID patients with fatty liver have more progressive changes in the chest CT scan. They have similar lung lesion distribution to non- fatty liver patients. Crazy paving lesions, septal thickening, Lymphadenopathy and plural effusion in fatty liver patients occurs in the same frequency as non-fatty liver patients

Keywords: CT scan, COVID 19, Fatty Liver.

Introduction

(COVID 19) is a respiratory disease caused by a member of the coronaviruses family, named (SARS-CoV-2), discovered in December 2019 in China. The majority of patients have cough, fever, myalgia, with or without dyspnea. Certain groups of patients developed severe illness. COVID-19 diagnosis depends on the real-time polymerase chain reaction (RT-PCR) test. Chest computed tomography (CT) is used for early detection, assess severity, and for follow-up of the patients. Typical findings appear when lung tissue early reacts to the insult as focal peripheral rounded ground-glass opacities (GGO), which may become more extensive, confluent, and may evolve to dense consolidation . Other less common CT scan findings include linear and curvilinear opacities, vascular enlargement, and bronchial dilation. Pulmonary nodules, lymph node enlargement, cavities, and pleural effusions are rare findings.

Findings associated with high mortality include extensive lung involvement and the presence of consolidations. As published in recent studies, patients with cardiovascular disease, diabetes, chronic kidney disease, and older age are at a higher risk of infection and prone to a graver outcome once infected. Non-alcoholic fatty liver disease (NAFLD) is another comorbidity that affects the severity of COVID-19. It is a chronic disease characterized by the presence of hepatic steatosis (intrahepatic lipid of more than 5% of liver weight) without a history of alcohol intake, with or without abnormal liver tests. It is the liver manifestation of the metabolic syndrome, which is characterized by
the presence of obesity, insulin resistance, hypertension, and hyperlipidemia. NAFLD has a doubtful association with infections; recent studies reported an association with community-acquired pneumonia and a higher risk of evolving to severe disease in COVID-19 patients. (19-23) The study aims to assess the association of the simple fatty liver disease with the severity of the chest CT scan findings in COVID-19.

Materials and Methods

1. Study population: It is a cross-sectional study done for 322 patients with COVID 19 at Azadi Teaching Hospital/Kirkuk city/Iraq, attending the CT scan unit for evaluation of their chests from March 2019- June 2019.

   Inclusion criteria: adult Patients with positive CT scan findings and nasopharyngeal RT-PCR tests were enrolling in the study.

   Exclusion criteria: Include patients with a known history of heart failure, pre-existing lung or liver disease, alcoholic persons, those with positive serology for hepatitis virus, and abnormal liver function tests. We collected the clinical information from the documentation of the clinicians.

2. Imaging: CT examinations without intravenous contrast medium achieved by a multidetector-row scanner (Siemens Brilliance), the energy level of 200-250 mA, and 120 kV. The patients imaged in the supine position. The slice thickness was 5 mm, images taken from the upper neck to the level of the umbilicus. The CT scan examinations in both lung and mediastinal windows evaluated by two radiologists with at least five years’ experience in CT scan imaging. Any differences of opinion resolved with consent. The CT examinations were done at 3-10 days from the onset of the symptoms.

3. CT interpretation: Liver density assessed to confirm the diagnosis of hepatic steatosis as liver attenuation less than 40 HU. (24) (Fig 1a)

   The CT COVID changes attenuation classified as pure GGO, predominantly GGO, or consolidation (Fig 1b), and pure consolidation attenuation, while the degree of lung involvement assessment done as the following: both lungs divided into six regions, three equal zones for each one: upper, middle, and lower zones. Each zone graded into less than 5% involvement, 5-25%, 25-50%, 50-75%, and > 75% lung involvement, then the average percentage is calculated for each patient. (25)

4. Statistical analysis: Continuous variables expressed as mean (± SD) and categorical variables as frequency (percentage). The lung lesion densities and extension compared between two groups differentiated according to their liver involvement by the fatty change. A probability (p) value of less than 0.05 was considered statistically significant. Version 17, SPSS software was used for statistical analyses.

This study approved by the Faculty of Medicine’s Research Ethics Committee/ Kirkuk University - Iraq. The individual data not explored in the study.

The patients gave verbal informed consent to participate in this study.

Figure 1. a, b. A 57-year-old woman with COVID-19, in the fatty liver group. Axial CT image (a) of the upper abdomen in abdominal window, average liver density is 11.9 HU (b) chest CT scan in the lung window, shows predominant consolidation in the both lower lobes.
Findings

Three hundred twenty-two patients (160 males and 162 females) with laboratory diagnosed COVID19 had positive CT scan findings. Their age range was 18-70 years, and the mean was 50.25 (± 13.1), 66 (20.5%) of them had fatty liver and 256 (79.5%) had normal liver density. (Table 1)

<table>
<thead>
<tr>
<th>Category</th>
<th>Fatty liver</th>
<th>Non fatty liver</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>66 (20.5%)</td>
<td>256 (79.5%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>50.92(± 11.44)</td>
<td>50.08 (± 13.49)</td>
<td>0.32 (t =0.46)</td>
</tr>
<tr>
<td>Weight</td>
<td>81.42 ± 14. 3</td>
<td>76 ± 8.4</td>
<td>&lt; 0.0001 (t = 3.97)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male N=160</td>
<td>33</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>Female N=162</td>
<td>33</td>
<td>129</td>
</tr>
</tbody>
</table>

1Using student t test, 2using chi-square test. The result is significant at p < 0.05. 3Number.

In the fatty liver group, the CT scan findings were pure GGO in 24 patients (36.36%), predominantly (more than 50%) GGO in 12 (18.18%), predominantly consolidation in 25 (37.87%), and Pure consolidation in 5 (7.57%). While in the non-fatty liver group, the findings were pure GGO in 75 patients (29.29%), predominantly GGO in 90 (35.15%), consolidation in 69 (26.95%), and Pure consolidation in 22 (8.59%). There is no statistical difference in pure consolidation and GGO between both groups in contrast to mixed density lesions which is predominantly GGO in the non-fatty liver group (P=0.003) and predominantly consolidation in the fatty liver group (P=0.04). (Table 2)

<table>
<thead>
<tr>
<th>Category</th>
<th>Pure GGO1</th>
<th>Predominantly GGO</th>
<th>Predominantly consolidation</th>
<th>Pure consolidation</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty-liver</td>
<td>24 (36%)</td>
<td>11 (16.66%)</td>
<td>26 (39.39%)</td>
<td>5 (7.5%)</td>
<td>66</td>
</tr>
<tr>
<td>Non fatty-liver</td>
<td>75 (30%)</td>
<td>90 (35%)</td>
<td>69 (26%)</td>
<td>22 (8%)</td>
<td>256</td>
</tr>
<tr>
<td>p-value</td>
<td>0.26</td>
<td>0.003</td>
<td>0.04</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

Using chi-square test. The result is significant at p < 0.05. 1Ground glass opacity.

Patients whose lungs involved by 5-25% were statistically more in the non-fatty liver group (P=0.00011), while more extensive involvement (25-50 %) was more in the fatty liver group (P=0.00001). While they had no difference in >5% and 50-75% lung involvement. (Table 3)
Table 3 relation between the lung lesions extension and the liver density.

<table>
<thead>
<tr>
<th>Category</th>
<th>Lungs percentage involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;5%</td>
</tr>
<tr>
<td>Fatty-liver</td>
<td></td>
</tr>
<tr>
<td>14 (21.21%)</td>
<td>27 (40.9%)</td>
</tr>
<tr>
<td>Non fatty-liver</td>
<td></td>
</tr>
<tr>
<td>54 (21.09%)</td>
<td>171 (66.79%)</td>
</tr>
<tr>
<td>Non fatty-liver</td>
<td></td>
</tr>
<tr>
<td>0.98</td>
<td>0.000116</td>
</tr>
</tbody>
</table>

Using chi-square test. The result is significant at p < 0.05.

In both fatty liver and non-fatty liver groups, the distribution of the lesion were mainly peripheral (63.63% and 56.25%), in lesser degree diffuse in (33.33%) and (35.15%) and to the least was central (3.03%) and (8.59%). The lesions in both groups were bilateral rather than unilateral (87.87% and 86.71%) versus (12.12%) and (13.28%). There was no significant difference in the lesion distribution in both groups. (Table 4)

Table 4 Distribution of lung lesions in the both groups.

<table>
<thead>
<tr>
<th>Category</th>
<th>Fatty-liver</th>
<th>Non fatty-liver</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral</td>
<td>42 (63.63%)</td>
<td>144 (56.25%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Central</td>
<td>2 (3.03%)</td>
<td>22 (8.59%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Diffuse</td>
<td>22 (33.33%)</td>
<td>90 (35.15%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Unilateral</td>
<td>8 (12.12%)</td>
<td>34 (13.28%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Bilateral</td>
<td>58 (87.87%)</td>
<td>222 (86.71%)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Using chi-square test. The result is significant at p < 0.05.

Other CT scan findings in the fatty-liver group were as the following: Crazy paving 24 (36%), Septal thickening 5 (5.57%), Plural effusion 22 (33.33%) and Mediastinal lymphadenopathy 1 (1.51%). While in non-fatty liver were 100 (39%), 10 (3.9%), 86 (33.56%), and 5 (1.95%) respectively. There was no significant difference in the incidence of these findings between the two groups.

There were no pericardial effusion, cavitation, neither nodule incidence in this study.

**Discussion**

In our study, 66 (20.5%) had fatty liver density, which approximates the global prevalence, which is about 25.24% (26). This group of people had a chance to develop more severe and extensive lung changes in COVID 19, as they had predominantly consolidative lesions, in contrast to the non-fatty-liver group who had GGO more. COVID 19 damaged the lungs of the Fatty liver group more extensively (25-50 %) in comparison to the non-fatty-liver group as their lungs involved more in (5-25%). It was consistent with the result of a study done in China by Kenneth I. Zheng et al who stated that (fatty liver patients that were obese had more severe COVID-19 disease) (27) as the fatty-liver group’s weight was significantly more in our study. (fatty-liver disease patients had significantly more recurrent
infections regardless of coexistent metabolic syndrome) a statement proved by William Nseir et al (22) The fatty liver patients had a higher risk of progressive COVID 19, a conclusion made out by Julie Lucifora et al. (28) This relation is probably due to the association of fatty liver change with low vitamin D serum levels, which in turn increases the susceptibility to infection and autoimmunity. (29, 30) In addition to the deficient innate immunity to the coronavirus representing by high M2 blood macrophages, which suppress the response to the infections. (31) In both groups, the distribution of the lung lesions was more frequently peripheral and bilateral, and to a lesser degree had a diffuse behavior, the least distribution pattern was unilateral and central lung involvement. The result was in line with much of the studies. (2, 6, 10, 12)

In both groups, like other papers, other CT scan findings were seen almost in the same percentage. About one-third of the patient had crazy paving lesions and septal thickening. Lymphadenopathy and plural effusion were relatively rare. (6, 10)

Limitation of the study: We did not consider the patients who may have received COVID 19 treatment, which might affect their CT scan findings.

**Conclusion**

COVID patients with fatty liver have more progressive changes in the chest CT scan.

The distribution of the lung lesions in both fatty and non- fatty liver are similar.

Crazy paving lesions, septal thickening, Lymphadenopathy and plural effusion in fatty liver patients occurs in the same frequency as non-fatty liver patients.

**Conflict of Interest:** None.

**Funding:** Self Funded.

**References**

13. Li K., Wu J., Wu F., Guo D., Chen L., and Fang Z. The clinical and chest CT features associated with


Studying the Relationship between the Lund Mackay Score and Response to Medical Treatment in Patients with Chronic Sinusitis

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Abstract

The present study was conducted to investigate the relationship between the Lund Mackay score and response to medical treatment in patients with chronic sinusitis. The present study was performed cross-sectionally on 150 patients referred to Khatam-ul-Anbia Hospital in Zahedan for whom the diagnosis of chronic sinusitis was confirmed and met the study criteria. Demographic data and clinical signs of patients were recorded for based on CT scan, The Lund Mackay score was evaluated. Patients were treated for 4 to 6 weeks and finally re-evaluated for symptoms and response to treatment. Data were analyzed using SPSS software version 24, McNemar test, Mann-Whitney and other necessary tests for descriptive statistics. A significance level of 0.05 was considered. 3.55% of patients were male (83 patients) and 7.44% were female (67 patients). The mean score of the Lund Mackay in the present study was 92.11 with a standard deviation of 2.3. Symptoms of olfactory dysfunction, sinus pain, nasal obstruction and nasal discharge showed significant improvement after treatment (p <05.0). Based on the results obtained at the end of the study, the presence of nasal polyps (p = 001.0) and nasal obstruction (p = 003.0), after the end of the treatment period, showed a significant relationship with the response to treatment. In general, this study suggested that the mean score of The Lund Mackay had a significant positive relationship with the severity of patients’ clinical symptoms and the severity of sinusitis. Therefore, it can be considered as a suitable criterion in diagnostic and therapeutic evaluations of patients.

Keywords: Chronic Sinusitis, the Lund Mackay Score, Medical Treatment

Introduction

Sinusitis Inflammation of one or more cavities of the paranasal sinuses, most often caused by viral infections of the upper respiratory tract (1). One of the most common reasons for patients to visit a doctor is Sinusitis. About 25 million people in the United States impose nearly $2 million directly to the medical system each year. Failure to correctly diagnose the disease and its causes or not paying attention to the patient’s underlying health problems which can be a contributing factor to this disease and also prescribing inappropriate drugs against sinusitis has led to the chronic type of the disease which means symptoms such as sinus tenderness. Or post-nasal drip for more than 12 weeks (2, 3). In cases of chronic sinusitis, its treatment requires more expensive antibiotics, and in some cases it does not respond to medical treatments and requires more aggressive treatments, such as endoscopic sinus surgery.

Due to the variety of treatment methods and the variable response of chronic sinusitis to treatment, it
seems necessary to follow the treatment process of patients and their response. Despite the centrality of clinical diagnosis in sinusitis, many physicians use CT scans to confirm the diagnosis of chronic sinusitis and determine the severity of the disease. CT scans are also used to determine anatomical abnormalities and to determine the roadmap for surgery. Therefore, CT scan is used as an essential component in the diagnosis and treatment of chronic sinusitis (4, 5).

It is often assumed that the symptoms in patients are related to objective scores obtained from the severity of the disease. However, the relationship between radiological findings and symptoms in sinusitis is discussed. Also in the treatment of chronic sinusitis, the predictive value of radiological scores in the description of symptomatic progression after surgery is still unknown. There are different staging systems in chronic sinusitis. Many studies in these cases have been performed using conventional radiography, CT, MRI, which have disadvantages, including superimposition of maxillary and facial structures on conventional radiography, the high-dose risk of radiation in CT; Lack of cortical bone imaging with MRI, therefore, this modality is not sufficient to show the sinus drainage system alone. In addition, it has disadvantages such as high price and insufficient access (6, 7).

The Lund Mackay scoring system became popular as a simple evaluation tool to facilitate treatment decisions in the mid-1980s (8). Considering the contradictory results of previous studies on the relationship between symptom severity and Lund Mackay radiological score, the present study attempts to find out the exact dimensions of the use of this scoring system in chronic sinusitis by examining this relationship in a significant population of chronic sinusitis patients.

**Material and Method**

**Participants:** The statistical population of this study includes patients who referred to Khatam Al-Anbia Hospital in Zahedan for treatment due to chronic sinusitis during 2016 to 2018 and met the necessary criteria. Ethical principles in accordance with the general guideline of ethics in medical sciences research with human subjects in the Islamic Republic of Iran were observed. Patients who had been treated for chronic sinusitis by an otolaryngologist were included. The patients must had had no structural abnormality in their CT scan such as retention cysts or nasal deviation. Exclusion criteria include patients with clinical symptom of acute sinusitis, patients with CT scans of symptoms such as retention cysts and patients with deviated septum without sinusitis and treatment protocol.

**Data collection:** Before beginning study, a questionnaire was completed that included questions about the patient’s history including age, gender, clinical symptoms of chronic sinusitis, and a history of illnesses. Then, this study was performed on 150 patients with chronic sinusitis who met the criteria of the study. CT scan of the paranasal sinuses was done for all patients. Based on the CT scan findings, The Lund Mackay score was calculated for each patient. In radiological sinusitis examination, considering the Lund Mackay score, a slight and severe increase in the thickness of the anterior and posterior Ethmoid sinuses, sphenoid, frontal, maxillary, and Ostitomeatal complex obstruction or air fluid surface as diagnostic signs and symptoms such as retention cyst and deviated septum without obvious sinusitis is considered as negative CT scan. Then, according to the Lund Mackay score, each scan with a score = 0 is normal and each scan with a score> 0 is abnormal (9). Also, at the beginning of the study, in addition to CT scan, DOTS score, which is a valid criterion for evaluating clinical symptoms, was calculated for patients. Then, patients were treated for 4 to 6 weeks, and re-examined for response to treatment and DOTS criteria. Ultimately, based on the treatment results, patients who did not respond well to treatment were recommended to do surgery.

**Statistical Analysis:** All statistical analyzes were performed using SPSS software version 24. Shapiro-Wilk test was used to examine the normal distribution of data. Considering the abnormal distribution, appropriate non-parametric tests were used in the relevant results. Patients were divided into three age groups: 1 (8 to 18), 2 (18 to 40) and 3 (over 40) years, and the Lund Mackay scoring was divided into 5 categories: 1 (8-5), category 2 (9-12), category 3 (13-16), group 4 (17-20) and group 5 (21-24) were divided.

**Findings**

**Patient baseline features:** The frequency distribution of age, gender and score of The Lund
Mackay is shown in Table 1. As shown in the table, 3.55% of patients were male (83 patients) and 7.44% were female (67 patients). 3.51% (77 people) were in the age group of 8 to 18 years, 30% (45 people) were in the age group of 18 to 40 years and 7.18% (28 people) were over 40 years old. Finally, the highest frequency distribution in terms of The Lund Mackay score is related to the score range 9 to 12 and includes 3.45% (68 patients) of patients. In this study, no cases of Wegener, immunodeficiency and migraine were found. Patients were divided into two groups: people with a history of allergies (67 people) and people with a negative history of the disease (83 people).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>55.3</td>
</tr>
<tr>
<td>Female</td>
<td>67</td>
<td>44.7</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-18</td>
<td>77</td>
<td>51.3</td>
</tr>
<tr>
<td>18-40</td>
<td>45</td>
<td>30.0</td>
</tr>
<tr>
<td>Over 40</td>
<td>28</td>
<td>18.7</td>
</tr>
<tr>
<td>The Lund Mackay score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>12.7</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>45.3</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>36.0</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

**The effect of treatment on DOTS clinical criteria:** The frequency of clinical criteria (DOTS criteria) before and after treatment is shown in Table 2. Changes in studied clinical variables, except nasal polyps, before and after treatment, showed a statistically significant difference (p < 0.05). Considering the information mentioned in the table, this difference in all these variables was a significant decrease in the frequency of the relevant symptom, but in the case of nasal polyps, the changes before and after treatment were not significant (p = 0.005).
Table 2: Frequency distribution of patients’ clinical symptoms before and after treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>percentage</td>
<td>Number</td>
</tr>
<tr>
<td>Olfactory dysfunctions</td>
<td>Have</td>
<td>51</td>
<td>34.0</td>
</tr>
<tr>
<td></td>
<td>Occasionally</td>
<td>54</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>Don’t have</td>
<td>45</td>
<td>30.0</td>
</tr>
<tr>
<td>Nasal polyps</td>
<td>Have</td>
<td>56</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>Don’t have</td>
<td>94</td>
<td>62.7</td>
</tr>
<tr>
<td>Sinus pains</td>
<td>Have</td>
<td>59</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td>Occasionally</td>
<td>61</td>
<td>40.7</td>
</tr>
<tr>
<td></td>
<td>Don’t have</td>
<td>30</td>
<td>20.0</td>
</tr>
<tr>
<td>Nasal obstructions</td>
<td>Have</td>
<td>78</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>Occasionally</td>
<td>60</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>Don’t have</td>
<td>12</td>
<td>8.0</td>
</tr>
<tr>
<td>Nasal discharges</td>
<td>Have</td>
<td>123</td>
<td>82.0</td>
</tr>
<tr>
<td></td>
<td>Occasionally</td>
<td>18</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Don’t have</td>
<td>9</td>
<td>6.0</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

The relationship between The Lund Mackay score and response to drug therapy in different age groups:

According to the findings shown in Table 3, in the age group of 18 to 40 years, the relationship between response to drug therapy and The Lund Mackay score was statistically significant (p = 0.002). Therefore, the average score of The Lund Mackay, in Patients who did not respond well to medication (requiring surgical treatment) (13.29±3.40) were significantly more likely than patients who responded well to medication (10.05±2.50). No significant relationship was found in the other 2 age groups.
Table 3: Correlation between The Lund Mackay score and treatment response in patients considering age

<table>
<thead>
<tr>
<th>Age</th>
<th>Response to treatment</th>
<th>The Lund Mackay score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>8-18 years</td>
<td>Appropriate response to drug therapy</td>
<td>11.50</td>
<td>2.87</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>11.90</td>
<td>4.20</td>
</tr>
<tr>
<td>18-40 years</td>
<td>Appropriate response to drug therapy</td>
<td>10.05</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>13.29</td>
<td>34.40</td>
</tr>
<tr>
<td>Over 40</td>
<td>Appropriate response to drug therapy</td>
<td>13.00</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>12.30</td>
<td>2.66</td>
</tr>
</tbody>
</table>

The relationship between The Lund Mackay score and response to drug therapy in different genders:
According to the findings shown in Table 4, the relationship between drug response and The Lund Mackay score in men was statistically significant (p =0.006). Therefore, the mean score of The Lund Mackay, in those patients who did not respond well to medication (requiring surgical treatment) (13.25 ± 3.09) were significantly higher than patients who responded well to medication (11.26±2.64). As can be seen in Table 4, the difference in scores in terms of response to treatment among women was not significant (p =0.109).

Table 4: Relationship between The Lund Mackay score and response to treatment considering gender

<table>
<thead>
<tr>
<th>Sex</th>
<th>Response to treatment</th>
<th>The Lund Mackay score</th>
<th>P value (Mann-Whitney)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Male</td>
<td>Appropriate response to drug therapy</td>
<td>11.36</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>13.25</td>
<td>3.09</td>
</tr>
<tr>
<td>Female</td>
<td>Appropriate response to drug therapy</td>
<td>11.05</td>
<td>3.11</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>12.50</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Relationship between The Lund Mackay score and response to drug therapy based on considering allergies: According to the findings shown in Table 5, in patients who did not report a specific disease history, unlike patients with allergies, the relationship between drug response and The Lund Mackay score was statistically significant (p = 0.026). Therefore, the mean score of The Lund Mackay was significantly higher in those patients who did not respond well to medication (requiring surgical treatment) (12.93±3.18) than in patients who responded well to medication (11.30±2.86).
Table 5: Relationship between The Lund Mackay score and response to treatment in patients considering disease type

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Response to treatment</th>
<th>The Lund Mackay</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Cough</td>
<td>Appropriate response to drug therapy</td>
<td>11.21</td>
<td>2.84</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>11.66</td>
<td>4.93</td>
</tr>
<tr>
<td>Allergy</td>
<td>Appropriate response to drug therapy</td>
<td>11.30</td>
<td>2.86</td>
</tr>
<tr>
<td></td>
<td>Requires surgery</td>
<td>12.93</td>
<td>3.18</td>
</tr>
</tbody>
</table>

**Relationship between The Lund Mackay score and response to drug therapy based on medical history:** Patients who did not report previous treatment, the relationship between response to drug therapy and the Lund Mackay score was statistically significant (p = 0.010), with the mean score of The Lund Mackay was significantly higher in patients who did not respond well to medication (requiring surgical treatment) (13.41 ±3.42) than in patients who responded well to medication (11.10 ±2.95). Therefore, in patients with a history of drug therapy (p = 0.163) or surgical treatment (p = 0.281), the difference in The Lund Mackay score with response to treatment was not statistically significant.

**The relationship between The Lund Mackay score and disease outcome status after the treatment:** All remaining symptoms at the end of the study are significantly correlated with the average score of The Lund Mackay. So, for all outcomes, the mean score of The Lund Mackay showed a significant increase in proportion to the severity of the clinical symptom (from high to low: permanent symptom, occasional symptom and no symptom, respectively) (P<0.05).

**Discussion**

Almost all studied clinical variables, before and after treatment, show a statistically significant difference. Symptoms of olfactory dysfunction, sinus pain, nasal obstruction and nasal discharge showed significant improvement after treatment, although in the case of nasal polyps, the changes before and after treatment were not significant. Patients who experienced outcomes such as sinus pain, nasal polyps, nasal obstruction, nasal discharge, and olfactory dysfunction had a higher mean, according to The Lund Mackay score, resulting in more severe sinusitis.

Also, all the remaining symptoms at the end of the study showed a significant relationship with the average score of The Lund Mackay. For all outcomes, the mean score of The Lund Mackay showed a significant increase in proportion to the increase in clinical symptom severity. For all outcomes the mean score of The Lund Mackay showed a significant increase in proportion to the increase in severity of clinical symptom.

Limited studies have been performed on the relationship between clinical findings and the Lund Mackay score. For instance, a study conducted by Safavi Naeini et al. on the diagnostic value of clinical signs and symptoms in the sinusitis diagnosis of 198 patients. This study was done according to the results of CT scan and considering the Lund Mackay system. In this study, it was found that patients suffering from nasal congestion and recurrent coughs had more severe sinusitis based on Lund Mackay score than those who did not (10). This finding is consistent with the results of the present study, which suggests the value of The Lund Mackay score in assessing the severity of sinusitis in patients. In a study by Hopkins et al. (2007), The Lund Mackay score was assessed in patients with chronic rhinosinusitis. In this...
multicenter study, it was suggested that those patients who had a higher mean score based on The Lund Mackay system were more likely to be candidates for surgery, and the higher the score, the more invasive surgery was used \(^{(11)}\). Also in the above study, patients were divided into two groups based on the status of nasal polyps: sinusitis patients with polyps and patients who had only sinusitis. In sinusitis patients without polyps, this CT scan score was significantly lower. According to a recent study that confirms our previous results and findings, The Lund Mackay score can be a good predictor of the severity of clinical symptoms and patients’ quality of life \(^{(12)}\).

However, studies have reported the results of the weakness of The Lund Mackay criterion in CT scan and also reported the Lund Mackay criterion’s weakness in predicting the patients’ clinical status with chronic rhinosinusitis, which highlights the importance of further studies for a more accurate view. For instance, in a study by Peter H. Hwang et al. on the association between the symptoms of chronic rhinosinusitis and the results of CT scans using the Lund Mackay, we found that out of 125 patients in the study, 115 patients had symptom criteria for CRS. However, 40 out of 115 patients had negative scans (Lund-Mackay = 0) despite having diagnostic criteria for rhinosinusitis. Out of 115 people, 75 had positive scans (Lund-Mackay > 1). Indicating poor convergence between chronic positive rhinosinusitis and CT positive \(^{(13)}\).

Limitations of this study are: Similar studies should be performed at longer time periods and with larger sample sizes for greater results certainty of evaluating changes in the Lund Mackay score in sinusitis patients. Second, it seems useful to evaluate the outcomes after surgery and compare it with the remaining outcomes after medical treatment. Third, the use of the Lund Mackay score classification in order to predict the response to treatment in patients and determine the appropriate treatment method in future studies seems necessary.

**Conclusion**

In summary, our study showed that there is a significant relationship between the Lund Mackay score based on CT scan findings and patients’ clinical status and treatment response. Therefore, this evaluation system can be used as an effective way to predict the prognosis of patients with chronic rhinosinusitis. However, current findings in this area are limited and further studies’ approval is needed. Another finding of this study, which seems to be new and important in its kind, is the great power of predicting clinical status with the help of CT scan in the age group of 18-40 years, male patients without a history of allergies, and without prior treatment.

**Ethical Clearance:** Obtained from institutional ethical committee

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Impact of Meningitis on Cognitive Skills and Development in Children

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Abstract

The present systematic review aims to investigate the impact of meningitis on the cognitive skills and development of children. In this systematic review, relevant studies were identified via searches through medical information databases of MEDLINE, PubMed, ISI, EMBASE, Cochrane with the keywords of Meningitis, Meningitis and Complications, Meningitis and Children, Meningitis and Intelligence Quotient (IQ), Meningitis and Development, and their derivatives. In this study, amongst 198 articles reviewed, 11 articles met the inclusion criteria. All studies, except one case, reported a decrease in growth and IQ in children who experienced meningitis. Based on the results of the present study, children who have experienced meningitis at a younger age are more prone to disability, growth retardation, and lower IQ than other children throughout the later years of their life.

Keywords: Meningitis, intelligence quotient (IQ), development, Children, Systematic Review

Introduction

Meningitis is the inflammation of the meninges or protective membranes that surround the brain and spinal cord (1, 2). The disease is usually caused by a bacterial or viral infection or, rarely, a fungal infection (1, 3). In the bacterial type, the three main pathogens are Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis (4) and in the viral type, enteroviruses, human parechoviruses and Chikungunya viruses are the causative agents of the disease (5). Mortality rate in viral meningitis is very low and most patients recover, but in bacterial meningitis, depending on the type of bacterium, it could kill 10% to 25% if left without appropriate treatment. Also, depending on the patient’s age, underlying disease, and surgery on the nervous system, different bacteria cause different symptoms in the patient. Bacterial meningitis is contagious and is caused by a specific bacterial infection that could be fatal if left untreated the number of leukocytes, cognitive impairment and infection with Streptococcus pneumonia were collected as prognostic factors in adults with bacterial meningitis. Meningitis, if left untreated, can lead to serious complications and even death. Therefore, early and accurate diagnosis is one of the research priorities of this disease. There are many methods available, such as examining the patient’s clinical symptoms, performing MRI and CT scans, biochemical blood tests, and examining the cerebrospinal fluid (6). Meningitis is a serious and dangerous disease that usually occurs quickly and unexpectedly. Although the disease affects different ages, the highest risk is for infants and young children (1). In children, the peak age of meningitis is 6 to 12 months, and 90% of cases occur in children under 5 years (7). The mortality rate of this disease is 2% in children and 20 to 30% in infants (8, 9). Symptoms of meningitis include fever, headache, refusal to eat or vomit, skin blemishes, drowsiness, photosensitivity, and neck spasms (10, 11). Pediatric imaging is one of the most indication of procedural sedation and analgesia. In the

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pediatric patient, performing CT scan is stressful and leads to increased mental stress, absence of cooperation with the staff, restlessness, and anxiety in the patient (12). However, any child with suspected meningitis and seizures should have an LP. All suspected children with meningitis must be closely monitored, whether LP or experimental treatment (6). Many pharmacologic and non-pharmacologic strategies to manage pain exist for pediatric pain treatment. Pharmacological and integrative non-pharmacological therapies has been indicated in acute and chronic pain treatment (13). The results showed that both pharmacological and non-pharmacological treatment methods in relieving and reducing pain and anxiety in a wide range of pediatric diseases (such as respiratory tract infections, burns, surgery, dental restorations) are effective (14). Although the clinical symptoms of most children who survive meningitis subside within a few weeks, some can lead to severe disorders such as cranial nerve dysfunction, hemiplegia, ataxia, hydrocephalus, physical disability, mental retardation, seizures, and visual and hearing impairments (15, 16). Overall intelligence, measured by IQ, is one of the most powerful predictors of a person’s future life, material well-being, and mental health. Even small deficiencies in intelligence affected by meningitis could impair a person’s chances of survival and academic achievement (17). Christie et al., In a systematic review of the effect of meningitis on children’s IQ and growth, reported that survival from bacterial meningitis had an adverse effect on children’s IQ and growth, while viral meningitis had no significant cognitive effects (17). The present systematic review also tries to investigate the effect of meningitis on IQ and development of children.

Materials and Methods

In this systematic review, relevant studies were identified via searches through medical information databases of MEDLINE, PubMed, ISI, EMBASE, Cochrane with the keywords of Meningitis, Meningitis and Complications, Meningitis and Children, Meningitis and Intelligence Quotient (IQ), Meningitis and Development, and their derivatives. The references in all articles found during the search process were evaluated, in order to include other possible sources in the study. Then, by studying the abstracts, the studies that were completely unrelated to the objective of this research were removed and the studies that were completely relevant or possibly related were recorded; also, by examining the full text of the articles, the articles that were completely relevant to the purpose of this research were selected. In the last stage, among the selected articles and based on the criteria of critical evaluation, the articles that had the inclusion criteria of the study were selected. It should be noted that all stages of evaluating the quality of articles were performed by two independent researchers.

Inclusion criteria: Laboratory meningitis proven to be caused by any infection (bacterial, viral or fungal), studies in which the results and consequences or complications of meningitis other than death or acute complications were evaluated, Time scale: Results or follow-ups reported 1 month after meningitis, Type of studies used: Prospective, retrospective, and case-control studies or cross-sectional studies on the consequences of meningitis, which were written in English, were used. Also, the articles in which the IQ or growth age or stage of development of the infant were evaluated using valid tools, were included in the present study; In this regard, Intelligence was considered as the average of full-scale IQ, Performance IQ (PIQ) or Verbal IQ (VIQ) as standard scores (mean = 100, SD = 15) or Ratios with low IQ (less than 70, i.e. 2 standard deviations (SD) below average), and Infant development was also considered as the desired result in growth performance in movements, language, or cognitive domains compared to standard data and DD measurements. Exclusion criteria: Studies in which acute complications were evaluated only 1 month after the onset of disease, were excluded. Articles published before 1955; Articles published in languages other than English; Articles about meningitis in people with the suppressed or weakened immune system. Screening and Data Extraction: Two trained authors performed search strategies. In the first stage, the titles and abstracts were reviewed for the selection of articles; in the next stage, the two authors independently reviewed the full text of the articles. Differences in findings were resolved through the criterion method of general conclusion of articles and were organized in this study.

Findings

In this study, 198 articles were reviewed. After removing 41 duplicates, 157 titles and abstracts were reviewed for content validity. After excluding 84 articles
due to content mismatch and differences in variables, 73 articles were registered (Figure 1).

![Diagram of the process of review and selection of articles]

Finally, 11 articles that met the inclusion criteria were selected, of which 4 articles were prospective cohort, 1 article was prospective, 1 article was a cohort study, 2 articles were retrospective cohort study, and in 3 articles the type of experiment was not specified.

All studies, except one case, reported a decrease in growth and IQ in children who experienced meningitis. Also, hearing loss or impairment in 36%, visual problems and blindness in 18% of cases, reduced ability to read and write in 18%, speech and language problems in 18%, neurological complications in 36%, seizures in 27%, Hydrocephalus in 9%, decreased mobility in 18%, cranial nerve palsy in 27%, and disability in 36% of these articles was reported.

**Discussion**

Meningitis affects different aspects of children’s development. Taylor et al. have reported that these children have poorer reading skills and need special supportive training; They also stated that the outcome of the disease is affected by the age and sex of the child and the socio-economic status of the family (18). In general, children with meningitis are at higher risk for disability, and these disabilities could include mild to moderate hearing loss and neurological or central auditory impairment, which adversely affects a child’s learning, academic performance, and behavior (19). Compared to control groups, these children showed significantly poorer results in the evaluations of intelligence and high-level neuropsychological skills and had more behavioral differences at home and at school. In addition, the risk of complications is higher in people who have experienced acute neurological complications during their illness (4). Grimwood et al. Reported that some low-level skills, such as attention, processing speed, and instant memory capacity, improve in 7- and 12-year assessments. However, high-level...
cognitive skills such as organizational skills, problem-solving, verbal fluency, and mental flexibility are still impaired in 12-year assessment. This may reflect a delay in the process of developing executive functions. According to Anderson et al., even 12 years after the illness, children with a history of meningitis experience neurobehavioral consequences. Although the risk of side effects is higher for children with acute neurological complications in bacterial meningitis, people with uncomplicated disease are also at risk, and they also have fewer functions than control children in a number of tasks and show a greater degree of disability. The data also show that the apparently normal survivors of meningitis are at a lower level of skill than their peers for a number of their intellectual, educational, and cognitive tasks.

Children with meningitis had a significant decrease compared to control children in terms of verbal intelligence, functional intelligence, and full-scale IQ. These children could not perform better than control children in tests of educational ability, visual skills, memory, learning, and executive skills. These differences between the children with meningitis and control children were approximately one-third of SD for each score. Although the mean IQ scores were close to the test standard, the results indicated a slight overall defect in meningitis survivors compared with control children. Infants with viral meningitis under 1 year, 1 to 6 years after the infection have smaller head circumference, lower IQ, and delayed language skills compared to control children. In a study by Christie et al., the results showed that survival from bacterial meningitis had a detrimental effect on IQ and growth, but there was no evidence that reports the efficacy of viral meningitis on IQ. The decrease in IQ in survivors of bacterial meningitis was approximately 5 degrees compared to the healthy control group and was equivalent to a 0.33% decrease in SD in IQ. Survivors of bacterial meningitis are five times more likely to develop mental disorders (IQ <70) than controls.

Briand et al. argued that a standard follow-up protocol could help create better rehabilitation protocols for children and parents. Neurological and neurophysiological follow-ups on a larger scale could be useful for the initial follow-up of children with learning disabilities. These follow-up tips may also be helpful for parents since they could help them understand the consequences of long-term complications.

## Conclusion

According to the results of the present study, children who have experienced meningitis at a younger age are more prone to disability, growth retardation, and lower IQ in later life than other children. Even the children who are apparently healthy survivors of the disease have shown developmental delays and problems in cognitive functions compared to healthy people. Families, school teachers, and health professionals play an important role in identifying or assisting people with learning and behavioral problems. Because the children without identifiable risk factors may have significant functional disabilities after meningitis, families and school teachers should be aware of possible language deficiencies and problems in understanding language-based content.

### Ethical Clearance:
Obtained from institutional ethical committee

### Source of Funding:
Self

### Conflict of Interest:
Nil

### References


The present study aimed to determine the relationship between subclinical hypothyroidism and diabetic retinopathy in patients with type 2 diabetes mellitus. In this cross-sectional analytical study, 150 patients with type 2 diabetes mellitus were assessed. The eye fundus examination was performed using fundoscopy to confirm or rule out retinopathy. Thyroid hormones were also measured by special kits to diagnose subclinical hypothyroidism. The criteria for diagnosis of this disorder include the high serum thyrotropin concentration (≥ 4mIU/L) plus normal serum free thyroxin levels. Statistical analyses were done using IBM-SPSS. In the study, 34.66% suffered retinopathy. A total of 24.0% suffered subclinical hypothyroidism with higher prevalence rate in those with retinopathy as compared with the group without retinopathy (p = 0.001). Those with diabetic retinopathy also experienced longer duration of diabetes than those without this event (p = 0.006). Analysis show a significant role for type 2 diabetes mellitus (OR = 1.121, P = 0.003), and high TSH level (OR = 1.342, P = 0.006) to predict diabetic retinopathy. This study showed an association between hypothyroidism, diabetes and increased TSH with retinopathy. Retinopathy is significantly predictable with the presence of subclinical hypothyroidism and longer duration of diabetes.

Keywords: Diabetes mellitus, Subclinical hypothyroidism, Retinopathy

Introduction

Type 2 diabetes mellitus is a major group of disorders characterized by varying degrees of insulin resistance, reduced insulin secretion and increased glucose production that affects around 285 million people worldwide in 2010, with a rapidly increasing prevalence around the world (1). The prevalence of this phenomenon is 0.2% in people under age 20, 11.3% in people over the age of 20 and 26.9% in people over 65 years of age. Meanwhile, due to an increase in the prevalence of obesity and a decrease in physical activity, the increase in the prevalence of type 2 diabetes mellitus has been higher than imagined (2). In diabetic patients, there are many acute and chronic complications that can be attributed to cardiovascular, renal, neurological, infectious and ocular complications (1, 3). Diabetic retinopathy is an ophthalmologic complication of diabetes mellitus and one of the causes of blindness in people with this disease (1, 4). Prolonged diabetes, poorly controlled blood glucose, advanced age, male gender, increased systolic blood pressure, consuming antihypertensive medications can increase the likelihood of diabetic retinopathy (5). Given that the prevalence of diabetes is increasing, the progression of diabetic retinopathy is also rising as one of the microvascular complications of diabetes mellitus (6). The prevalence of retinopathy, non-proliferative retinopathy and proliferative retinopathy was 3.1%, 1.1% and 0.1% in the general population and 23%, 19.1% and 8.2% in the diabetic population, respectively. Since the most effective treatment for diabetic retinopathy is to prevent it, the importance of identifying risk factors and the
causes of increased prevalence of diabetic retinopathy is clarified (2, 4). The thyroid plays an important role in the body’s metabolism (7) and recently suggested that the prevalence of people with thyroid disorders, especially subclinical hypothyroidism, was significantly higher in people with type 2 diabetes mellitus. Subclinical hypothyroidism is an asymptomatic stage of hypothyroidism characterized by elevated serum thyrotropin levels and serum free thyroxine levels (8). Patients with subclinical hypothyroidism have an endothelial dysfunction, which may lead to dysfunction of the small veins as the main fundament of retinopathy (8, 9). Some Studies demonstrated association between high thyroid stimulating hormone (TSH) levels and an increased risk of diabetic (10, 11) and some studies do not show this relationship (12). For this reason, in many studies, it is recommended that people with thyroid disorders, especially subclinical hypothyroidism, should be screened for retinopathy so that the condition can be given to health authorities in a controlled manner. Therefore, due to the high prevalence of diabetes and hypothyroidism and various complications due to the lack of control of diabetes, especially retinopathy, and the possible association of these two diseases with diabetic retinopathy, the present study aimed to determine the relationship between subclinical hypothyroidism and diabetic retinopathy in patients with type 2 diabetes mellitus.

Materials and Methods

Study design: In this cross-sectional analytical study, 150 patients with type 2 diabetes mellitus who referred to the internal clinic of Kowsar Hospital of Semnan between 2013 and 2014, with the preservation of inclusion and exclusion criteria were assessed. The sampling method was convenience sampling. Furthermore, data were collected through a questionnaire and Para clinical results.

Inclusion and exclusion criteria: The inclusion criteria were having type II diabetes mellitus, disease duration ranged 2 to 5 years, serum hemoglobin A1C 6.5% or higher, lack of clinical manifestations of thyroid disorders including fatigue, malaise, skin dryness, puffiness of face and hands, lack of laboratory findings related to thyroid disorders including raised TSH level along with reduced free T4, and conscious consent to participate in the study. In this regard, those with the history of cancer, liver or kidney disorders, chronic infections, using the drugs affecting thyroid functions (such as glucocorticoids, oral contraceptives, or NSAIDS) were all excluded from the study.

Study protocol and Biologic samples: After taking the medical records including demographics, medical history and medications, a clinical examination was performed to determine height, weight and blood pressure. Also, thyroid stimulating hormone (TSH), free thyroxine (free T4), hemoglobin A1C (HBA1C) and fasting blood glucose (FBS) were measured at the hospital laboratory using the special kits. TSH and T4 levels were measured by radioimmunoassay technique. HBA1C was measured by high-performance liquid chromatography (HPLC). FBS Fasting blood glucose was also measured by a glucose oxidase method. The eye fundus examination was performed using fundoscopy. The information was included in the data collection forms, which was previously prepared by the researcher for this purpose. This work continued until the sample volume was completed.

Data collection: Venous blood samples were collected in test tubes containing clot activator, immediately stored on ice, and—one hour after collection—centrifuged at 4000 rpm for 10 minutes. Plasma were separated and stored at −80°C through the hour to be analyzed. Those with normal free thyroxine (FT4) and an increased TSH (≥4 μIU/ml) level were diagnosed with SCH. Digital retinal photographs (two eyes × two fields), taken using a TRC-NW7SF (Topcon, Tokyo, Japan) non-mydriatic camera at 45°, were examined independently by two qualified retinal photography graders following quality assurance protocols. The severity of diabetic retinopathy was graded based on the international clinical diabetic retinopathy severity scale. data of patients and laboratory results were recorded in a pre-designed information form and finally entered for statistical analysis.

Statistical analysis: Descriptive analysis was used to describe the data, including mean ± standard deviation (SD) for quantitative variables and frequency for categorical variables. Chi square test, independent t test were used for comparison of variables. The correlation between quantitative variables was tested by the Pearson’s correlation test. For the statistical analysis, the
statistical software IBM SPSS Statistics for Windows version 25.0 was used. P values <0.05 were considered statistically significant.

**Findings**

In this study, 150 patients with type 2 diabetes mellitus were analyzed. The mean age of patients was 57.94 ± 9.68 years including 93 men (62.0%) and 57 females (38.0%). Of the participants in the study, 52 (34.66%) suffered retinopathy. None of the patients has overt or clinical hypothyroidism. Comparing baseline characteristics between the patients with and without retinopathy showed no difference in patients’ age (p = 0.232), gender (p = 0.329), body mass index (p = 0.431) (Table 1).

<table>
<thead>
<tr>
<th>Item</th>
<th>Group with retinopathy</th>
<th>Group without retinopathy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age subgroups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>5 (9.6)</td>
<td>25 (25.5)</td>
<td>0.232</td>
</tr>
<tr>
<td>50-59 years</td>
<td>19 (36.5)</td>
<td>41 (41.8)</td>
<td></td>
</tr>
<tr>
<td>60-69 years</td>
<td>16 (23.1)</td>
<td>21 (21.5)</td>
<td></td>
</tr>
<tr>
<td>≥ 70 years</td>
<td>12 (23.1)</td>
<td>11 (11.2)</td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>34 (65.4)</td>
<td>59 (60.2)</td>
<td>0.329</td>
</tr>
<tr>
<td>Disease duration</td>
<td>13.73 ± 1.06</td>
<td>9.03 ± 0.51</td>
<td>0.006</td>
</tr>
<tr>
<td>5-10 years</td>
<td>25 (48.1)</td>
<td>70 (71.4)</td>
<td></td>
</tr>
<tr>
<td>11-20 years</td>
<td>18 (34.6)</td>
<td>23 (23.5)</td>
<td></td>
</tr>
<tr>
<td>21-30 years</td>
<td>8 (15.4)</td>
<td>5 (5.1)</td>
<td></td>
</tr>
<tr>
<td>&gt;30 years</td>
<td>1 (1.9)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.21 ± 0.58</td>
<td>25.63 ± 0.46</td>
<td>0.431</td>
</tr>
<tr>
<td>&lt; 25 kg/m2</td>
<td>22 (42.3)</td>
<td>44 (44.9)</td>
<td></td>
</tr>
<tr>
<td>25-29.9 kg/m2</td>
<td>19 (36.5)</td>
<td>33 (33.7)</td>
<td></td>
</tr>
<tr>
<td>≥ 30 kg/m2</td>
<td>11 (21.2)</td>
<td>21 (21.4)</td>
<td></td>
</tr>
</tbody>
</table>

Results showed that subclinical hypothyroidism is associated with retinopathy and this relationship is statistically significant. No significant correlation systolic blood pressure (p = 0.365), diastolic blood pressure (p = 0.685), the level of fasting blood sugar (p = 0.401), and HBA1C level (p = 0.466), while those with diabetic retinopathy experienced longer duration of diabetes than those without this event. (13.73 ± 1.06 years versus 9.03 ± 0.51 years, p = 0.006). The mean level of TSH in the patients with and without retinopathy was 4.68 ± 0.96 and 2.13 ±0.18 with significant difference (p = 0.006) (Table 2).
Table 2 Comparing Biochemical characteristics

<table>
<thead>
<tr>
<th>Item</th>
<th>Group with retinopathy</th>
<th>Group without retinopathy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean systolic blood pressure, mmHg</td>
<td>127.00 ± 2.00</td>
<td>123.44 ± 1.31</td>
<td>0.365</td>
</tr>
<tr>
<td>Mean diastolic blood pressure, mmHg</td>
<td>78.60 ± 1.15</td>
<td>78.79 ± 0.85</td>
<td>0.658</td>
</tr>
<tr>
<td>Fasting blood sugar, mg/dl</td>
<td>159.52 ± 13.06</td>
<td>148.81 ± 5.62</td>
<td>0.401</td>
</tr>
<tr>
<td>&lt; 100 mg/dl</td>
<td>11 (21.2)</td>
<td>14 (14.3)</td>
<td></td>
</tr>
<tr>
<td>100-126 mg/dl</td>
<td>11 (21.2)</td>
<td>29 (29.6)</td>
<td></td>
</tr>
<tr>
<td>&gt;126 mg/dl</td>
<td>30 (57.7)</td>
<td>55 (56.1)</td>
<td></td>
</tr>
<tr>
<td>Mean hemoglobin A1C</td>
<td>7.89 ± 0.20</td>
<td>7.77 ± 0.16</td>
<td>0.466</td>
</tr>
<tr>
<td>Mean TSH level</td>
<td>4.68 ± 0.96</td>
<td>2.13 ± 0.18</td>
<td>0.006</td>
</tr>
<tr>
<td>Prevalence of hypothyroidism</td>
<td>36 (24.0)</td>
<td>22 (42.3)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

A total of 36 patients (24.0%) suffered subclinical hypothyroidism with higher prevalence rate in those with retinopathy as compared with the group without retinopathy (42.3% versus 14.3%). However, there was no difference in the level of T4 between the patients with and without diabetic retinopathy (1.10 ± 0.04 ng/dL versus 1.20 ± 0.02 ng/dL, p = 0.222). In order to investigate the relationship between the mentioned variables on retinopathy, logistic regression analysis showed that type 2 diabetes mellitus was significantly associated with retinopathy (OR = 1.121, 95%CI: 1.041 – 1.208, P = 0.003), and high TSH level (OR = 1.342, 95%CI: 1.086 – 1.657, P = 0.006) to predict diabetic retinopathy (Table 3).

Table 2: Multivariable logistic regression model to predict diabetic retinopathy

<table>
<thead>
<tr>
<th>Item</th>
<th>Beta</th>
<th>Wald</th>
<th>P value</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.014</td>
<td>0.378</td>
<td>0.540</td>
<td>0.969</td>
<td>1.063</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.048</td>
<td>0.013</td>
<td>0.909</td>
<td>0.418</td>
<td>2.174</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>0.115</td>
<td>9.119</td>
<td>0.003</td>
<td>1.041</td>
<td>1.208</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.028</td>
<td>2.827</td>
<td>0.093</td>
<td>0.995</td>
<td>1.063</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.024</td>
<td>0.850</td>
<td>0.357</td>
<td>0.927</td>
<td>1.028</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.063</td>
<td>1.883</td>
<td>0.170</td>
<td>0.973</td>
<td>1.165</td>
</tr>
<tr>
<td>Fasting blood sugar</td>
<td>0.001</td>
<td>0.027</td>
<td>0.870</td>
<td>0.994</td>
<td>1.007</td>
</tr>
<tr>
<td>Hemoglobin A1C</td>
<td>-0.025</td>
<td>0.028</td>
<td>0.867</td>
<td>0.724</td>
<td>1.312</td>
</tr>
<tr>
<td>Free T4</td>
<td>-0.530</td>
<td>0.484</td>
<td>0.486</td>
<td>0.133</td>
<td>2.616</td>
</tr>
<tr>
<td>TSH</td>
<td>0.294</td>
<td>7.454</td>
<td>0.006</td>
<td>1.086</td>
<td>1.687</td>
</tr>
</tbody>
</table>
Discussion

In our study, it was found that subclinical hypothyroidism is directly and significantly associated with retinopathy. Also, retinopathy was directly and significantly associated with Type 2 diabetes mellitus and TSH levels. Coherent research has shown patients with subclinical hypothyroidism have an endothelial dysfunction, which may lead to inappropriate operation of small veins increasing the likelihood of retinopathy \(^8,^9\). The association between thyroid hormone levels and retinopathy has been observed in other studies \(^10,^11,^13\). For this reason, in many studies, it is recommended that people with thyroid disorders, especially subclinical hypothyroidism should be screened for retinopathy. In study on 1581 subjects with normal thyroid function, the results showed that TSH levels had a positive correlation with insulin resistance and, as a result, with diabetic retinopathy \(^14\). In another study conducted on the Beijing Tongren Hospital of China on 1,117 patients with type 2 diabetes mellitus, it was found that the prevalence of diabetic retinopathy in people with subclinical hypothyroidism was higher than those with normal thyroid \(^9\). Also, although the level of fasting blood glucose and hypertension are known to be two factors affecting diabetes \(^15-17\), Yuedong Hu (2015) noted that these two factors had no meaningful relationship with diabetic retinopathy \(^18\). Moreover, in a study of 11140 patients with type 2 diabetes mellitus, the results showed that control of blood pressure below the normal level, even for 5 years, had no significant effect on the prevalence of diabetic retinopathy \(^19\), which was similar to our findings. In a study conducted by Javadi et al. (2009) in Saudi Arabia, more than 50% of people with diabetic retinopathy were older than 60 years of age \(^20\), which was also similar to our study results. Similarly, in a study by Khalid Al-Rubeaan et al. (2015), unlike our study, the prevalence of diabetic retinopathy in males was more than females \(^21\). The reason for this was the lower number of males in our study than the similar studies, which reduced the likelihood of diabetic retinopathy in men. Additionally, obesity is a problem because of the direct relationship with diabetes \(^22,^23\). In this regard, Studies noted that the BMI of diabetic individuals had a meaningful relationship with diabetic retinopathy \(^21,^24,^25\), but in our study, there was no significant relationship between BMI and risk for retinopathy.

One of the most important limitations of this study was its cross-sectional nature that limited the outcome analysis. Another limitation in this study was the relatively small number of statistical samples that should be cautious about generalizing its outcomes to other individuals and populations. Also, in this study, the definition and division of obesity has been done based on the BMI of individuals; while this index does not differentiate between muscle distribution and fat distribution. Even the distribution of fat in different parts of the body could have changed the risk of developing diabetes in different individuals, as the results of various studies indicate that the distribution of fat levels in the upper and lower abdomen, the risk of Metabolic diseases, especially diabetes, are increased more than those who have a fat mass in the lower parts of the body \(^26-29\).

One of the other limitations of this study was the lack of examination of a number of diabetes-related factors mentioned in other studies, including smoking, as well as exercise and physical activity, which, of course, due to the limited means of measuring this study. Also, the treatment of diabetics (insulin or oral medication) was another issue that could not be verified due to the limitations of this study.

Conclusion

Subclinical hypothyroidism is associated with diabetic retinopathy in type 2 diabetic patients. There is a positive correlation between level of TSH and intensity of diabetic retinopathy in type 2 diabetics with subclinical hypothyroidism. Also, subclinical hypothyroidism was one factor that significantly increased the risk of diabetic retinopathy. Therefore, considering the high prevalence of diabetic retinopathy and its relation to the subclinical hypothyroidism, planning for the education of patients at risk for diabetes or diabetic patients regarding long-term complications of diabetes, especially retinopathy is essential.

Ethical Clearance: Obtained from institutional ethical committee

Source of Funding: Self

Conflict of Interest: Nil
References


A Survey of Knowledge Level about Pediatric oral/dental Health among Pediatricians

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Abstract
Pediatricians play a central role in children’s health care and are the first line of care for oral/dental conditions. The level of pediatrician knowledge about oral/dental health is not accurately determined. In this cross-sectional study, a previously adapted questionnaire was used to evaluate the level of oral/dental health knowledge among pediatricians affiliated with medical universities in Tehran, Iran. The data were summarized through descriptive statistics and central index tests. In this study, 263 pediatricians (49.4% male) completed the questionnaire. The mean age was 43.9 years. Only 17 individuals (6.5%) had completed a course of oral/dental health during their residency. The mean score was 5.9 ± 1.7. Questionnaire scores were similar between male and female pediatricians. Time from graduation and completion of an oral/dental health course was not associated with better performance according to the questionnaire. The level of knowledge about oral/dental health among pediatricians is inadequate. Most pediatricians have not completed a training course on this matter. These findings highlight the need for integrated training programs to improve pediatrician knowledge about oral/dental health.

Keywords: Knowledge Level, Pediatric oral/dental Health, Pediatricians, Medical Universities.

Introduction
Oral/dental diseases such as dental carries and periodontal conditions are among the most common chronic conditions in children, which begin during the first few years of life and could be best avoided in infancy and childhood (¹). Negligence of such disorders could lead to dental and gingival complications, tooth loss, distorted self-image, and decreased quality of life (²). Prevention is considered as the most effective method to avoid such complications and to improve oral health. The increasing prevalence of dental carries and gingivitis among children in developing countries makes them important subjects in health-care policy (³,⁴). Children are an important target for oral health preventive interventions; hence, providing consistent measures of prevention and education in this age group is of paramount importance (⁵,⁶).

Pediatricians are the first-line in children’s health-care, meaning their familiarity with oral/dental health subjects in providing evidence-based care and education to families can promote prevention of oral/dental diseases (⁷). A few studies in this field have demonstrated inadequate levels of oral/dental health training among pediatricians (⁸). Previous studies were conducted among Iranian medical interns and pediatric residents, which revealed substandard oral/dental knowledge level among these groups (⁹,¹⁰). There is limited evidence concerning the sufficiency of pediatricians’ oral/dental knowledge. In the present study, we sought to determine the level of oral/dental health knowledge among pediatricians in Tehran.

Material and Methods
Study design and participants: In this cross-
sectional descriptive study, between 2018 and 2019, a group of pediatricians who practiced in Tehran and other Iran city were asked to complete a questionnaire regarding oral and dental hygiene among children. Participant selection was done through convenience sampling.

**The questionnaire:** The questionnaire used in this work was adopted from previous studies, previously conducted pilot surveys, and was approved by dentistry professors in all universities involved (9,10). There were four initial questions about the participant’s age, sex, the number of years passed since their pediatrics residency program graduation, and whether they have taken part in any specific course on oral/dental hygiene during their residency. These were followed by 15 multiple-choice questions covering pediatric oral and dental hygiene, tooth-brushing, routine dentistry evaluations, fluoride use, and tooth development. Each question was assigned a score of 1 if the provided answer was correct, and 0 in case of an incorrect answer. The performance of each individual was evaluated according to the sum of the scores and was designated as “weak” for a score of 1-5, “moderate” for 6-10, and “good” for 11-15.

**Data analysis:** Sex and participation in an oral / dental course are demonstrated as number (percentage). Age, years since graduation, and questionnaire scores are demonstrated as mean ± standard deviation. Comparison of categorical and continuous variables was made between males and females using Chi-Squared test and independent samples T test, respectively. An association between two continuous variables was tested by Pearson correlation.

**Findings**

Overall, 263 participants who completed the questionnaire were included in the study. Among the participants, 130 (49.4%) were males, and the mean age was 43.9 ± 7.3 years. The mean time from residency graduation was 8.2 ± 4.1 years and 17 individuals (6.5%) stated that they had completed a course of oral/dental hygiene during their residency.

The mean score was 5.9 ± 1.7 among the studied population. Frequency of correct responses are demonstrated in Table 1 and visualized. The minimum score was 1 and the maximum score was 11. The classification of pediatricians according to questionnaire scores are shown in Table 2.

**Table 1. Frequency of correct answers provided to each question by pediatricians.**

<table>
<thead>
<tr>
<th>Question</th>
<th>Correct answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>151 (57.4%)</td>
</tr>
<tr>
<td>2</td>
<td>93 (35.4%)</td>
</tr>
<tr>
<td>3</td>
<td>35 (13.3%)</td>
</tr>
<tr>
<td>4</td>
<td>54 (20.5%)</td>
</tr>
<tr>
<td>5</td>
<td>216 (82.1%)</td>
</tr>
<tr>
<td>6</td>
<td>69 (26.2%)</td>
</tr>
<tr>
<td>7</td>
<td>159 (60.5%)</td>
</tr>
<tr>
<td>8</td>
<td>59 (22.4%)</td>
</tr>
<tr>
<td>9</td>
<td>115 (43.7%)</td>
</tr>
<tr>
<td>10</td>
<td>112 (42.6%)</td>
</tr>
<tr>
<td>11</td>
<td>88 (33.5%)</td>
</tr>
<tr>
<td>12</td>
<td>84 (31.9%)</td>
</tr>
<tr>
<td>13</td>
<td>172 (65.4%)</td>
</tr>
<tr>
<td>14</td>
<td>98 (37.3%)</td>
</tr>
<tr>
<td>15</td>
<td>34 (12.9%)</td>
</tr>
</tbody>
</table>

Data are presented as number (percentage)
Table 2. Classification of the oral/dental knowledge of pediatricians according to the questionnaire score.

<table>
<thead>
<tr>
<th>Score</th>
<th>Number (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak</td>
<td>112 (42.6%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>149 (56.7%)</td>
</tr>
<tr>
<td>good</td>
<td>2 (0.8%)</td>
</tr>
</tbody>
</table>

Questionnaire scores were not different between male and female pediatricians (5.7 in males versus 6.0 in females; p = 0.19). Older pediatricians had higher scores but the association of time from graduation with oral/dental hygiene knowledge nearly missed signficancy (p = 0.051). The scores were similar between those who had completed a course on oral/dental hygiene during their residency and those who had not. (p = 0.168)

Discussion

According to these results, the level of pediatricians’ knowledge about oral/dental health is far from sufficient. Furthermore, only a small fraction of pediatricians reported participation in a course on oral/dental hygiene during their training. Gender and time of graduation had no significant association with the questionnaire score. Importantly, pediatricians are generally the first-line in the care of children who may have oral/dental conditions. Pediatricians are the physicians who refer children to dentists and lack of knowledge and confidence to perform a directed oral/dental exam can lead to under-appreciation of such conditions in the pediatrics practice.

A previous study of medical interns in Tehran with the same questionnaire showed that oral/dental health knowledge is inadequate among trainees and concluded that new training programs should be incorporated in the medical curriculum to address this issue. The mean score of participants was 4.87 and age, sex, and duration of training did not show any association with the score (9). Another study with a similar questionnaire was conducted among pediatrics residents of Tehran which demonstrated an insufficient level of oral/dental health knowledge in residents. Moreover, none of the participating pediatric residents had been through a course focusing on oral/dental health. The mean score 6.43 and only 27.3% had a high enough score to be considered as good dental knowledge (10).

Various reports from other studies point to the suboptimal attention towards oral/dental health of children. In a Canadian study, the authors reported that only 1.8% of pediatricians could answer all the oral health-related questions correctly, and 73.9% of them reported regularly inspecting patients’ teeth. The study concluded that lack of appropriate knowledge impedes high-quality dental care for children (12). A study from Belgium reported that pediatricians’ knowledge about oral health was insufficient and highlighted the need to improve this knowledge among physicians involved in preventive care of children. In this study, 71% of pediatricians reported to have taken part in some sort of training course for oral/dental health, and most relied on information brochures (13).

Herndon et al. studied the level of oral health knowledge in pediatricians and general practitioner and concluded that pediatricians performed better in this regard; however, both groups had suboptimal knowledge and improvement was needed. They found no direct association between training for oral/dental disease prevention and performing the recommended practices. Nevertheless, training was associated with increased confidence relating to dental issues (14). A study from India reported that while 58% of general dental practitioners were aware of early childhood caries, only 2% of medical practitioners had knowledge about this condition. Among medical professionals, older age was associated with better dental health knowledge (15).

In a study from Shiraz, the knowledge, attitude, and practice regarding dental health was assessed among general practitioners and pediatricians. A self-completed questionnaire was used for this study. There was not a significant difference between general practitioners and pediatricians in knowledge, attitude, and practice. Physicians with higher working hours, and those who worked in government-administered centers with high workloads demonstrated a higher level of knowledge and better practice. On the other hand, there was no
association between the time passed from graduation and their performance (16). These data point out that overall, pediatrician knowledge about oral/dental conditions can be improved, which could in turn lead to more focused prevention strategies with reduction in the burden of pediatric and adult oral/dental diseases and conditions. Such improvement in pediatrician knowledge is specifically important in the developing countries. It should be noted that our findings are limited by the small sample size, which was due the reluctance of specialists to complete the questionnaire.

Conclusion

According to the present study, the level of pediatricians’ knowledge about oral/dental health in children is far from appropriate. Most pediatricians in this study did not receive any oral/dental health training during their residency. Due to their central role in children’s care and the importance of oral/dental conditions in this age group, providing focused training in this subject for pediatricians seems necessary.

Ethical Clearance: Obtained from institutional ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References

Clinical Characteristics and Short-Term Outcomes of Patients with Severe COVID-19

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Abstract

We report the demographic characteristics, laboratory findings, vital signs and outcomes of 19 laboratory-confirmed COVID-19 patients who admitted to ICU; from 15 Feb to 15 March 2020 in Arak, Markazi Province, Iran. Data showed that the most common laboratory findings on first day of ICU admission, were leukocytosis (52.6%), neutrophilia (73.6%), lymphopenia (100%), anemia (100%), hyperglycemia (78.95), increased levels of INR (100%); AST (66.66%), ALT (50%) and LDH (100%). 36.36% and 45.45% of patients had elevated total and direct bilirubin, respectively but all of patients had normal range of ALK-p (Table 1). In term of vital signs (3 first days); SBP, DBP and PR did not have significant changes but O₂ saturation increased from 82.9 % to 93 % and the number of mechanical ventilated patients increased from 36.8% to 73.3%

Keywords: COVID-19, Arak, Laboratory findings, Vital signs

Introduction

Corona virus can damage the respiratory system and about 26% of patients with Covid-19 need intensive care unit (ICU) care (¹). At presents, Covid-19 has a substantial morbidity and mortality and is known as a global threat (²). Due to the lack of information, we report the demographic characteristics, laboratory findings, vital signs and outcomes of 19 laboratory-confirmed COVID-19 patients who admitted to ICU; from 15 Feb to 15 March 2020 in Arak, Markazi Province, Iran.

Materials and Method

Our data were obtained from two hospitals affiliated to Arak University of Medical Sciences in the center on Iran. Oropharyngeal samples were collected and tested by COVID-19 RT-PCR at Arak laboratory for symptomatic cases. RT-PCR was done according to the same protocol described by National Institute for Viral Disease Control and Prevention (China). Data were analyzed using Stata software version 13.

Findings

The mean age was 71.53 (S.D: 10.87, range: 52-92) years that 52.63% of them were male, 78.95% of individuals have past medical history (DM; 56.25%, HTN=IHD= HLP; 15.78%). The most common clinical and computed tomography (CT) manifestations were shortness of breath 76.92%, fever 69.23%, cough 61.54%, myalgia 30.77% and bilateral mixed ground glass opacities with consolidations 57.89%. The most common laboratory findings on first day of ICU admission, were leukocytosis (52.6%), neutrophilia (73.6%), lymphopenia (100%), anemia (100%), hyperglycemia (78.95), increased levels of INR (100%); AST (66.66%), ALT (50%) and LDH (100%). 36.36%
and 45.45% of patients had elevated total and direct bilirubin, respectively but all of patients had normal range of ALK-p (Table 1). In term of vital signs (3 first days); SBP, DBP and PR did not have significant changes but O2 saturation increased from 82.9 % to 93 % and the number of mechanical ventilated patients increased from 36.8% to 73.3% (Table 2).

**Table 1. The mean (S.D.) of laboratory tests on first day for COVID-19 patients**

<table>
<thead>
<tr>
<th>Variables</th>
<th>First day</th>
<th>Normal range</th>
<th>Increased</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC</strong></td>
<td>9.42 (3.7)</td>
<td>3.5-.9.5*10⁹/L</td>
<td>17(52.6%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td><strong>PMN</strong></td>
<td>89.27 (5.3)</td>
<td>1.5-6.5*10⁹/L</td>
<td>14 (73.6%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td><strong>Lymph</strong></td>
<td>5.70 (3.7)</td>
<td>1.1-3.2*10⁹/L</td>
<td>0 (0.0%)</td>
<td>19(100.0%)</td>
</tr>
<tr>
<td><strong>Hb</strong></td>
<td>12.55 (1.9)</td>
<td>12-17g/L</td>
<td>0 (0.0%)</td>
<td>19(100.0%)</td>
</tr>
<tr>
<td><strong>Plt</strong></td>
<td>194.6 (80.5)</td>
<td>125-350*10⁹/L</td>
<td>2 (10.5%)</td>
<td>6 (31.5%)</td>
</tr>
<tr>
<td><strong>Na</strong></td>
<td>136.6 (2.9)</td>
<td>137-140 mmol/L</td>
<td>1 (5.2%)</td>
<td>9 (47.3%)</td>
</tr>
<tr>
<td><strong>K</strong></td>
<td>3.94 (0.35)</td>
<td>3.8-4.8 mmol/L</td>
<td>1 (5.2%)</td>
<td>9 (47.3%)</td>
</tr>
<tr>
<td><strong>PTT</strong></td>
<td>36.2 (4.7)</td>
<td>31.5-43.5s</td>
<td>2 (10.52%)</td>
<td>4 (21.0%)</td>
</tr>
<tr>
<td><strong>PT</strong></td>
<td>15.8 (1.5)</td>
<td>9.4-12.5 s</td>
<td>18 (94.7%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td><strong>INR</strong></td>
<td>1.43 (0.2)</td>
<td>0.9-1 index</td>
<td>19 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>FBS</strong></td>
<td>175.7 (82.2)</td>
<td>100-110 ng/dl</td>
<td>15 (78.9%)</td>
<td>2 (10.5%)</td>
</tr>
<tr>
<td><strong>BUN</strong></td>
<td>53.3 (18.6)</td>
<td>17-45mg/l</td>
<td>11 (57.89%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>1.41 (0.57)</td>
<td>0.8-1.3mg/l</td>
<td>6 (31.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>120.2 (263.7)</td>
<td>7-37 U/L</td>
<td>6 (50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>108.5(193.6)</td>
<td>10-40 U/L</td>
<td>8(66.66)</td>
<td>0(0%)</td>
</tr>
<tr>
<td><strong>Bili T</strong></td>
<td>13.5 (43.3)</td>
<td>Up to 1.2mmol/L</td>
<td>4 (36.36%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Bili D</strong></td>
<td>0.40 (0.25)</td>
<td>Up to 0.4 mg/dL</td>
<td>5 (45.45%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Alb</strong></td>
<td>2.84 (1.10)</td>
<td>28.9-36.0g/L</td>
<td>0 (0.0%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td><strong>CPK</strong></td>
<td>235.5 (125.1)</td>
<td>39-308 U/L</td>
<td>2 (33.33%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>LDH</strong></td>
<td>820.4 (309.1)</td>
<td>125-243U/L</td>
<td>8 (100%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>
Table 2. The mean of vital signs in the 3 first days of admission (n=19)

<table>
<thead>
<tr>
<th>Variables</th>
<th>SBP</th>
<th>DBP</th>
<th>PR</th>
<th>RR</th>
<th>Ventilated (%)</th>
<th>O2 sat</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day</td>
<td>108.9 (13.6)</td>
<td>73.6 (12.1)</td>
<td>98 (12.5)</td>
<td>23.9 (6.6)</td>
<td>7 (36.8%)</td>
<td>82.9 (13.9)</td>
</tr>
<tr>
<td>2nd day</td>
<td>111.3 (12.6)</td>
<td>74.8 (12.4)</td>
<td>101.6 (15.4)</td>
<td>19.8 (1.1)</td>
<td>9 (52.9%)</td>
<td>93.5 (3.4)</td>
</tr>
<tr>
<td>3rd day</td>
<td>110 (9.2)</td>
<td>73.6 (11.1)</td>
<td>100.7 (14.3)</td>
<td>20.5 (0.57)</td>
<td>11 (73.3%)</td>
<td>93 (4.1)</td>
</tr>
</tbody>
</table>

The mean of Hospitalization, ICU stay, time from hospital to ICU admission and intubation period were 8.36 (S.D; 4.70, range; 1-17), 5.73 (S.D; 4.66, range; 1-16), 2.57 (S.D; 3.0, range; 0-10 days) and 4.52 (S.D; 5.48, range; 0-17) respectively. Besides, only one patient extubated from mechanical ventilation (intubation period; 6 days) and discharged from ICU. Among died patients, super infection (e.g. bacterial, fungal and etc) weren’t seeing in any patients. Of these patients, 1 patient (5.26%) has been discharged and eight patients (42.1%) are active patients and case fatality rate (CFR) was estimated 52.63% (95%CI: 29.2-74.9%).

**Discussion**

Most of the COVID-19 patients who admitted to ICU were old and male and had liver injury (hepatocellular form), and also like available studies on COVID-19 patients (3-5), presented with leukocytosis, neutrophilia, lymphopeni and high level of AST, ALT and bilirubin. Liver injury in sever patients with COVID-19 is higher than mild one (6) and it seems this virus affected liver and maybe through this way induces sever or lethal condition. That’s why, above factors which can consider as prognostic factors in COVID-19 patients. Besides, the most common initial symptoms and CT findings of them were shortness of breath, fever, cough and bilateral mixed GGO with consolidation which confirmed by available studies. CFR in COVID-19 ICU patients was estimated 52.63% and the number of mechanical ventilated patients increased that maybe indicate an exacerbation of pulmonary involvement and can be a cause of high mortality in ICU. In the end, according to past evidences that have showed the SARS-Cov is naturally neuroinvasive (7-9) and induces neuron death in animal model (10), also according to limitation in data about neurological problems in COVID-19 patients, the sudden cardiac arrest of these patients and no responsivenes of them to cardiopulmonary resuscitation (CPR) can because of central nerves system involvement. This our hypothesis needs to future wide investigation in COVID-19 patients.

**Conclusion**

The mortality rate is too high in ICU admitted patients and the results suggested that past medical history has an important role on COVID-19 mortality rate.

**Ethical Clearance:** Ethical approval for the study was provided by the Ethical Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1398.333).

**Source of Funding:** Vice chancellor of research and technology of the Arak University of Medical Sciences was funded the study. The funder has no role in data collection, analysis, interpretation and manuscript drafting

**Conflict of Interest:** Nil

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Assessment of Diabetes Patient Adherence to Dietary Recommendation in Diabetics Center in Al-Najaf City

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1M.Sc. Adults Nursing, Faculty of Nursing, University of Kufa, 2M.Sc. Maternal and Newborn Health Nursing

Abstract

Objective: This study aimed to assess of diabetes patient adherence to dietary recommendation in Diabetics center in Al-Najaf city and to find out the relationship between the diabetic patient adherence to dietary recommendation and their demographic and clinical data.

Methodology: Descriptive analytic design was carried out to assess of diabetics patient adherence to dietary recommendation in Al-Najaf City/ Health Directorate of Al-Najaf Al-Ashraf / Al-Sadder Medical City / Al-Najaf Center for Diabetes and Endocrine. The study was carried out from February 1st, 2018 up to March 28th, 2018. A non-probability (accidental) sampling technique was used consisting of (100) female and male those who visit Al-Najaf Center for Diabetes and Endocrine. Data collected through using of a well-designed questionnaire consist of three parts: Part I: Socio-demographic Characteristics, Part II: Clinical Data: consists of (6) items and Part 111: Patient adherence to dietary recommendation. This part of the questionnaire consists of (16) items.

Conclusion: It is concluded that the Majority of the stay sample have fair adherence to diabetics dietary recommendations.

Recommendation: Based on study conclusion, its need for a good relationship between the patient and health-care provider in order to provide more understanding and knowledge about the disease and its non-medical management and diabetic’s patients need dietary recommendations with means of education, such as published materials with regard to dietary regimen.

Keywords: Diabetes, Patient, Dietary Recommendation, Diabetics Center

Introduction

Diabetes mellitus (DM) consist an enormous public health problem globally, associated with high morbidity and mortality [1]. Normally, a certain amount of glucose circulates in the blood. The major sources of this glucose are absorption of ingested food in the gastrointestinal tract and formation of glucose by the liver from food substances. Diabetes mellitus (DM) is a chronic progressive metabolic disorder characterized by hyperglycemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin hormone [2]. The exact mechanisms that lead to insulin resistance and impaired insulin secretion in type 2 diabetes are unknown, although genetic factors are thought to play a role. Regardless of the specific cause, the destruction of the beta cells results in decreased insulin production, unchecked glucose production by the liver and fasting beta cells cannot keep up with the increased demand for insulin, the glucose level rises and type 2 diabetes develops. Despite the impaired insulin secretion that is characteristic of type 2 diabetes, there is enough insulin present to prevent the breakdown of fat and the accompanying production of ketone bodies [3]. The treatment of diabetes should start with non-pharmacological therapies such as lifestyle interventions. Diet plays a major role in the therapeutic strategy to keep patients with diabetes in good glycemic control and prevent micro and macro vascular complications [4]. According to the American Diabetes Association (ADA) and the European Association for the study of Diabetes
(EASD) prescribed dietary recommendations for the treatment of this disease since many years [4]. Type 2 Diabetes is defined as chronic hyperglycemia resulting from either decreased insulin secretion, impaired insulin action or both in the absence of autoimmune destruction of the pancreatic beta cell. Classically, type 2 diabetes occurs in the older, obese patients in the setting of strong family histories of diabetes and in association with other components of the metabolic syndrome [5]. Unhealthy practices and perception for diabetic patients can lead to increase the effect of the disease and complications which can be prevented. The diabetic complications such as nephropathy and retinopathy and neuropathy can affect the quality of life of the patients and can lead to a significant effect on patient’s quality of life and productivity [6]. Healthy dietary habits and lifestyle modifications the cornerstones of type 2 diabetes prevention and management [7]. Adherence to lifestyle modification recommendations lessens the disease burden and reduces the morbidity and mortality associated with type 2 diabetic complications [8].

Methodology

Design of the study: Descriptive analytic design was carried out to assess of diabetics patient adherence to dietary recommendation in Al-Najaf City/ Health Directorate of Al-Najaf Al-Ashraf / Al-Sadder Medical City / Al-Najaf Center for Diabetes and Endocrine.

Study objectives: This study aimed to assess of diabetics patient adherence to dietary recommendation in Diabetics center in Al-Najaf city and to find out the relationship between the diabetic patient adherence to dietary recommendation and their demographic and clinical

Duration of the study: The study was carried out from February 1st, 2018 up to March 28th, 2018.

Sample of the Study: A non-probability (accidental) sampling technique was used consisting of (100) female and male those who visit Al-Najaf Center for Diabetes and Endocrine. The Study Instrument: Data collected through using of a well-designed questionnaire consist of three parts: Part I: Socio-demographic Characteristics: This part consists of (7) items, including (gender, age, and marital status, level of education, occupational status, residency and socio-economic status) and Part II: Clinical Data: consists of (6) items, including (duration of disease since diagnosis, treatment, health education regarding self-care activities, complications, smoking and body mass index). Part 111: Patient adherence to dietary recommendation. This part of the questionnaire consists of (16) items, the items include assessing patients’ adherence to dietary recommendation.

Ethical consideration: This is essential value and should be obtained before collecting the information, to respect the patient’s dignity and values. The investigator can achieve this permission from the moral committee at the Nursing Department in the faculty of Nursing / University of Kufa. Also another agreement from Al-Najaf Al-Ashraf Health Directorate / Al-Sadder medical city / Al-Najaf Center for Diabetes and Endocrine. In addition to above, the researcher told each participant that this is an involuntary work, and they can leave any time even the interview process uncompleted.

Questionnaire Validity: The questionnaire validity faces validity for the initial developed instrument which is specified through panel of (5) experts (4) Experts from faculty of Nursing/University of Kufa (with experience of > 5 yrs at their jobs field). Who were asked to review the instrument, and to inspect relevancy, clarity, and sufficiency of the questionnaire to measure the concept of interest. The data was collected through the applying of the developed questionnaire with aid of structured interview technique with the subjects as they are individually interviewed. The data collection process started from March 1st, 2018 to March, 20th, 2018.

Statistical Analysis: In this study, the data were analyzed by using of (SPSS) program version 19 (Statistical Package for Science Service), and the statistical package (Excel 2013).
## Result

Table (1) shows the Study Sample Demographic Data and their discussion

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Rating and intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td></td>
<td>46.0%</td>
</tr>
<tr>
<td>Male</td>
<td>54</td>
<td></td>
<td>54.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Age / years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>40.00 - 44.00</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>45.00 - 49.00</td>
<td>12</td>
<td></td>
<td>12.0%</td>
</tr>
<tr>
<td>50.00+</td>
<td>86</td>
<td></td>
<td>86.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>86</td>
<td></td>
<td>86.0%</td>
</tr>
<tr>
<td>Single</td>
<td>4</td>
<td></td>
<td>4.0%</td>
</tr>
<tr>
<td>Divorced</td>
<td>9</td>
<td></td>
<td>9.0%</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Levels of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>31</td>
<td></td>
<td>31.0%</td>
</tr>
<tr>
<td>Able to read and write</td>
<td>16</td>
<td></td>
<td>16.0%</td>
</tr>
<tr>
<td>Primary school</td>
<td>20</td>
<td></td>
<td>20.0%</td>
</tr>
<tr>
<td>Intermediate school</td>
<td>14</td>
<td></td>
<td>14.0%</td>
</tr>
<tr>
<td>High school</td>
<td>8</td>
<td></td>
<td>8.0%</td>
</tr>
<tr>
<td>Institute and college</td>
<td>11</td>
<td></td>
<td>11.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Occupational status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>1</td>
<td></td>
<td>1.0%</td>
</tr>
<tr>
<td>Government employee</td>
<td>12</td>
<td></td>
<td>12.0%</td>
</tr>
<tr>
<td>Retired</td>
<td>12</td>
<td></td>
<td>12.0%</td>
</tr>
<tr>
<td>Free job</td>
<td>19</td>
<td></td>
<td>19.0%</td>
</tr>
<tr>
<td>Housewife</td>
<td>45</td>
<td></td>
<td>45.0%</td>
</tr>
<tr>
<td>Jobless</td>
<td>11</td>
<td></td>
<td>11.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Economic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>27</td>
<td></td>
<td>27.0%</td>
</tr>
<tr>
<td>Barely sufficient</td>
<td>47</td>
<td></td>
<td>47.0%</td>
</tr>
<tr>
<td>Insufficient</td>
<td>26</td>
<td></td>
<td>26.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>Residency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>84</td>
<td></td>
<td>84.0%</td>
</tr>
<tr>
<td>Rural</td>
<td>16</td>
<td></td>
<td>16.0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Freq= Frequency, % = Percent.
According the above table, the majority (54.0%) of sample were male, (86.0%) of patients were (50) years old and more. (86.0%) of patients were married. Concerning the level of education (31.0%) of sample was illiterate. (45.0%) of patient housewife. (47.0%) of sample economic status were barely sufficient. (84.0%) of patients live in urban area.

**Table (2) statistical analysis of diabetic patient’s clinical data and their discussion**

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Rating and intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of disease / years</td>
<td>1-3</td>
<td>23</td>
<td>23.0%</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>11</td>
<td>11.0%</td>
</tr>
<tr>
<td></td>
<td>7-9</td>
<td>10</td>
<td>10.0%</td>
</tr>
<tr>
<td></td>
<td>10 And More</td>
<td>56</td>
<td>56.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>Treatment</td>
<td>Oral hypoglycemia</td>
<td>34</td>
<td>34.0%</td>
</tr>
<tr>
<td></td>
<td>Injection</td>
<td>14</td>
<td>14.0%</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>3</td>
<td>3.0%</td>
</tr>
<tr>
<td></td>
<td>All of them</td>
<td>11</td>
<td>11.0%</td>
</tr>
<tr>
<td></td>
<td>Oral hypoglycemia &amp; Injection</td>
<td>13</td>
<td>13.0%</td>
</tr>
<tr>
<td></td>
<td>Oral hypoglycemia &amp; Diet</td>
<td>17</td>
<td>17.0%</td>
</tr>
<tr>
<td></td>
<td>Injection &amp; Diet</td>
<td>8</td>
<td>8.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>Education regarding self-care</td>
<td>Yes</td>
<td>72</td>
<td>72.0%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>28</td>
<td>28.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>16</td>
<td>16.0%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>84</td>
<td>84.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
<tr>
<td>BMI</td>
<td>Obese</td>
<td>37</td>
<td>37.0%</td>
</tr>
<tr>
<td></td>
<td>Overweight</td>
<td>38</td>
<td>38.0%</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>25</td>
<td>25.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
**Table (3) Overall assessment of diabetic patients’ adherence to dietary recommendations**

<table>
<thead>
<tr>
<th>Overall assessment for patients’ adherence</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good adherence</td>
<td>5</td>
<td>5.0%</td>
</tr>
<tr>
<td>Fair adherence</td>
<td>91</td>
<td>91.0%</td>
</tr>
<tr>
<td>Poor adherence</td>
<td>4</td>
<td>4.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table (1) reveals that the majority (56.0%) of patient’s duration of disease were 10 years and more. The most of patients used tablets route to taking drugs (34.0%). About (72.0%) of patients received self-care education. (84.0%) of sample are not smoking. Around (38.0%) of patient were overweight.

Table (3) show, that majority (91.0%) of sample with fair adherence to dietary recommendations

**Table (4) Relationship between the diabetic patients’ adherence to dietary recommendations and their demographic and clinical data**

<table>
<thead>
<tr>
<th>Demographic And Clinical Data</th>
<th>Chi-Square Value</th>
<th>Df</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.099</td>
<td>2</td>
<td>.952 NS</td>
</tr>
<tr>
<td>Age / years</td>
<td>6.412</td>
<td>6</td>
<td>.379 NS</td>
</tr>
<tr>
<td>Marital Status</td>
<td>8.045</td>
<td>6</td>
<td>.235 NS</td>
</tr>
<tr>
<td>Levels of education</td>
<td>16.241</td>
<td>10</td>
<td>.093 NS</td>
</tr>
<tr>
<td>Occupation</td>
<td>24.608</td>
<td>10</td>
<td>.006 HS</td>
</tr>
<tr>
<td>Economic Status</td>
<td>0.469</td>
<td>4</td>
<td>.976 NS</td>
</tr>
<tr>
<td>Residency</td>
<td>0.326</td>
<td>2</td>
<td>.849 NS</td>
</tr>
<tr>
<td>Duration Of Disease</td>
<td>4.672</td>
<td>6</td>
<td>.587 NS</td>
</tr>
<tr>
<td>Treatment</td>
<td>32.755</td>
<td>14</td>
<td>.003 HS</td>
</tr>
<tr>
<td>Education</td>
<td>3.835</td>
<td>2</td>
<td>.147 NS</td>
</tr>
<tr>
<td>Complications</td>
<td>67.251</td>
<td>46</td>
<td>.022 S</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.326</td>
<td>2</td>
<td>.849 NS</td>
</tr>
<tr>
<td>BMI</td>
<td>2.963</td>
<td>4</td>
<td>.564 NS</td>
</tr>
</tbody>
</table>

Related to table (4) there were good relationships between the patient (occupation, type of treatment and complications) with their adherence to dietary recommendations.
Discussion

According to (Table 1) in the results, the study shows that the entire study sample (54%) are male. The finding is consistent with results of [9] which mentioned in their study result that majority of the study subject were male. Regarding their age, the majority (86%) of the research samples are at age group of (50) yrs and more. This result is in agreement with other study [10] in their study found that a third of the participants, (26.9%) aged 50 to 59 years. Concerning the marital status the majority (86%) of the study subjects are married. This finding reinforced with the study result of [11] which mentioned majority of the study sample were married. In related to the level of education the most of the study samples (31%) are illiterate while regarding the diabetic patient occupational status, about (45.0%) are house wife. These results matching with the study result of [8]. Concerning socio-economic status, about half of the sample (47%) reveals their economic status that is barely sufficient, while concerning the residency the study result show that highest percentage (84%) of the study sample are lived in urban area. These result reinforced with the study of [2] which mentioned that 80% of people with diabetes live in low- and middle-income countries

Concerning duration of disease in the (table 2), the higher percentage (56%) is for those who are suffering from the diabetic for period from 10 years and more. This result comes along with the findings of other study which is carried out by [12].

Regarding the type of treatment, the results of the study display that the highest percentage (34%) of the study subjects are with oral hypoglycemia treatment. This result matching with the study result of [9] which mentioned the majority of participants were taking oral hypoglycemic medications. The present study shows that in regards to the education related to self-care, the most of the study subject (72%) are not received education related to self-care. This finding may related to the lack of nursing education and instruction to the patient about the dietary and exercise recommendation for the management of the diabetic disease and to reduce the complications related to disease. Related to the smoking, the majority of the study samples (84%) are not smoker. These findings matching with the study done by [13] who represents the majority of their study sample were not smoker. In regard to the study subject body mass index, the most of the study subject (38%) are overweight. These result agreements with the study finding done by [14] that represent the majority of their sample were overweight. Concerning the complications related to disease, the highest percentage of the study sample suffers from Neuropathy & Eye problems.

According to the above (table 3) in the result, the overall assessment of diabetic’s patient adherence to dietary recommendation is fair. This outcome is reinforced by a study done by [12] who concluded in their study results that the level of adherence to dietary recommendations in the study group was moderate

Table (4) in the result shows that there are high significant relationship between two items (occupation and treatment ) and patient’s adherence to dietary recommendation at p-value less than 0.05, in addition there were significant relationship between the complication related to the disease and patients adherence to dietary recommendation at p-value less than 0.05. While there are non-significant relationship between the other items of diabetic patients demographic and clinical data and their adherence to dietary recommendation at p-value more than 0.05

Conclusions:

It is concluded that the most of the research sample are male and their dominant age are within 50 years and more, the most of them are illiterate. It was summaries that the most of the study sample taking oral-hypoglycemia medication, most of them not received education related to the disease and their dietary recommendation and in regard to their BMI it’s concluded that most of them are overweight. It is concluded that the Majority of the stay sample have fair adherence to diabetics dietary recommendations. It is concluded that there are significant relationship between the patient (type of treatment, occupation and complication related to disease) and their adherence to dietary recommendations.

Recommendations:

Based on study conclusion, its need for a good relationship between the patient and health- care provider in order to provide more understanding and knowledge
about the disease and its non-medical management. Encourage the diabetic patients who visit the diabetic center to follow the healthy dietary recommendations. Diabetic’s patients need dietary recommendations with means of education, such as published materials with regard to dietary regimen. It is recommended to design proper booklet by the diabetic’s center to guide the visitors about the non-medical management of diabetic’s disease.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Nursing and all experiments were carried out in accordance with approved guidelines.

References

5. Guidelines for Clinical Care Ambulatory, Management of Type 2 Diabetes Mellitus, UMHS, 2017.
7. Bisiriyu G. Non-adherence to Lifestyle Modification Recommendations (diet and exercise) among type 2 Diabetes Mellitus Patients attending Extension Clinic in Gaborone, Botswana. Published Thesis. Faculty of Medicine at the University of Limpopo (Medunsa Campus), Republic of South Africa, 2008;13-15.
The Application of an Indigenous Polymer for the Plastination of Teaching Anatomical and Biological Specimens

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Abstract

Plastination was fabricated in 1978 by Dr. Gunther Von Hagens at the University of Heidelberg, Germany, which kept for the good conservation of anatomical and biological material. The present goal was to utilize a cost-effective plastination polymer as compared to the standard S10 technique using silicone polymers. The S10 is the original silicone polymer used for the preparation of plastinated specimens and whole dissecting body.

Specimens were fixed in formalin 10%, dehydrated and decreasing in acetone, and at last, impregnated by local commercial unsaturated polyester resin and ultimately hardening at 50 °C temperature.

The plastinated specimens were clean, durable, odorless, portable and non-toxic, it can be kept for long durations without any changes. The usage of widespread S10 silicon method is high costs so with the aid of using indigenous chemicals it is possible to produced low costs anatomical models for education and for studying anatomy.

Keywords: polyester resin, education, impregnation, formalin, plastination.

Introduction

In the plastination technique, the water and lipids in biological and anatomical tissues are substituted by a curable polymer. The hardener polymer give, dry, odorless, durable, and devoid of noxious effects of formalin specimens. The first innovate of the plastination at the Anatomical Institute of Heidelberg university, Germany by Dr. Gunther Von Hagens in 1979.

In the last years plastination a large evolution take place in which gross anatomical and biological educations.

The plastination laboratory was designed and established at the College of Veterinary Medicine, University of Basra since 2013 and has produced good quality plastinated specimens.

Generally the polymers that used are the silicone, epoxy or polyester resins which hold the specimens in a dry, odorless state with minimal aftercare.

The main disadvantage of using formalin for fixation and preservation of tissues are that they are fripper when its handled, with difficulty transportation and troubles of spillage. In addition to its toxicity. However, formalin is still the main preservative of tissues due to its low costs, the plastination technique applied polymers which are forcefully impregnated into the tissues to get stable and free from deterioration specimens. They can easy handled and not brittle like the formalin preserved tissues.

The methods in plastination includes of four steps – fixation, dehydration, forced impregnation in a vacuum and hardening, many curable polymers used in this process.

An indigenous polyester resin was used in this procedure was cheaper, with the same efficiency in comparison with standard silicon S10, and it was a motivating materials when compared to other teaching aids like wet specimens, glass and wood models. The plastination is being used as a method of preserving specimens and anatomical organs at the College of...
Materials and Methods

In the college of veterinary Medicine, University of Basrah, Indigenous polymer has been convenient for preservation of anatomical specimens and organs since 2013.

The procedure includes, Fixation, Dehydration, Forced impregnation, and hardening

Fixation

Anatomical specimens were fixed before plastination to avoid corruption and prevention the action of other enzymes. Whole fixation of the sample were very important to the final quality of a plastinated organ, the plastinated specimens that have been fixed in 10% formalin for several days according to its size, this gave the tissues steadiness and stops autolysis. The fixed organs were washed in clean water to rub from excessive fixative.

Dehydration

After formalin fixation the specimens were put in acetone for dehydration. There were three changes of acetone, each for three weeks, In this step, the acetone displaced gradually instead the water in the tissues. The volume of acetone should was 5-10 times than the volume of the specimens. Acetonometer (Fig1) was used every day to monitor the specific gravity of acetone and the level of dehydration. When the concentration stabilized at 98% the dehydration became complete.

Forced Impregnation

Acetone saturated specimens were transfer into a large vacuumed chamber (Fig2) and submerged in locally obtained polyester resin (Fig3) at freezer temperature 10-25°C (Fig3) gradually the vacuum raised till reached 5 mm Hg using vacuum pump and vacuum gauge (Fig4). Pressure can be controlled by adjustment of a shutoff valve in the line between the pump and chamber to stabilized the level pressure at the chamber. Impregnation checking by showing the acetone bubbles released from the polymer surface due to its lower boiling point +56°C, the indication to be complete when the acetone bubbles stopped liberation the specimens are taken out of the vacuum chamber. The excess resin is exsiccating.

Heat hardening

After complete impregnation the specimens cured in a heat treatment at 50°C temperature for curing, final curing in an oven take about 7-10 days depending at the size of the specimens.

Results

The plastination method can be used for whole body or organs, in human and animals, for Anatomical, pathological and surgical specimens in addition to biological samples such as parasites and insects (Fig:5). The advantage of this methods to obtained odorless dry and sturdy actual biological specimens (Fig:6), Non-toxic and can handles easy without gloves, preservation anatomical specimens without the usual problems such as moistened specimens, mold and not required special storage climate. By this method the students conceived to use of Plastination specimens in the dissection room to be helpful to anatomical education and it’s a good and enjoyable method improved to understanding the anatomy for students and provides an additional tool for long-term maintenance for anatomical education.

Fig.1: The specific gravity of acetone and the level of dehydration monitor by acetonemeter
Fig. 2: a vacuum chambers for forced impregnations

Fig. 3: polyester resin

Fig. 4: vacuum pump for dehydration and forced impregnation
Discussion

The first foundation of plastination laboratory in Iraq and production of resin specimens at the college of veterinary medicine, University of Basrah. These characteristics are similar to 9 which mentioned that the plastination laboratory in the College of Veterinary Medicine at the University of Basra, Iraq, was designed and built to use the standard S10 method of plastination.

Plastination technique can be applied for whole body or organs, in human and animals, for anatomical, pathological and surgical specimens in addition to biological samples such as parasites and insects, that in comparable with plastination has become an important means of preservation of organs, for well dissected specimens 8. The specimens obtained in our methods were dry, odorless and non-toxic that can handled easy without gloves, its in agreement with 11 plastination technique has yielded dry, odorless and durable plastinates which are useful as an adjunct for demonstration at museums. Plastication was good and enjoyable method improved to understanding the anatomy for students and museums 6 explained that the specimens serves as a great aid for understanding anatomy of different organs.
in their original form with any disturbance of smell.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Basrah and all experiments were carried out in accordance with approved guidelines.

**References**


Evaluations of Inflammatory Status in Chronic Renal Failure Patients Undergoing Hemodialysis and Conservative Treatment

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Abstract

Introduction: - Chronic inflammation is a basic feature of end stage kidney disease and is associated with increasing risk of mortality and morbidity in patients undergoing dialysis. The aim: - the study amid to evaluate white blood cells and IL-6 in patients undergoing hemodialysis and without hemodialysis. Methods and Subjects: - The study was designed on 106 patients with chronic renal failure age range (20-69 years). The patients divided in two groups according to treatment, 40 patients with conservative treatment and 66 patients with hemodialysis treatment. IL-6 were determined by using a commercially available ELISA Micro wells kit. Total white blood cells count and differential count were determined by using an automated hematology autoanalyzer XT2000i. Results: - showed a significant (p<0.05) decrease of total white blood cells count, neutrophils, lymphocytes, monocytes, eosinophils and basophils in hemodialytic group when compared with conservative group. But IL-6 levels was higher significantly (p<0.01) in hemodialytic group when compared with conservative group. Conclusion: - hemodialysis session impaired immune system and activated anti-inflammatory factors.

Keywords: - Hemodialysis, Conservative treatment, Chronic inflammation

Introduction

Chronic kidney disease (CKD) is a life-threatening disorder often related with hypertension, kidney dysfunction, progression to kidney fibrosis, and eventual chronic renal failure [1,2]. Chronic inflammation is a basic feature of end stage kidney disease and is associated to an increasing risk of mortality and morbidity in patients under hemodialysis [3, 4, 5]. Patients with hemodialysis treatment, have an increased susceptibility to infectious diseases compared with healthy subjects [6, 7]. White blood cells play important function in host defense mechanism against infectious diseases [8].

The three mechanisms stimulation of the pro-inflammatory system may be occurs in hemodialytic patients: inflammatory response stimulated by bio-incompatible dialysis membranes, contact to local fistula and graft infections and contact to contaminated dialysate containing cytokine-inducing substances such as endotoxins [5] On the other hand, membrane adsorption of cytokines and clearance through HD session may be change circulating cytokine levels [9]. Some of inflammatory marker could be used in medical practice that include interleukin-6 secreted from activated endothelial cells, monocytes, adipocytes, fibroblasts and macrophages as a result to several stimuli, such as bacterial endotoxins, IL-1b, tumor necrosis factor (TNF-a), oxidative stress and physical exercises. The main effects of IL-6 are result of its level in the circulation and take place at distinct positions and far from its origin [10]. The possible causes of raised IL-6 levels in ESRD diseased people may be belong to the losing of renal function and some factors are related with hemodialysis [11].
The aim: The aim of this study was to evaluate white blood cells and IL-6 in patients undergoing hemodialysis and without hemodialysis.

Subjects and Methods

The study was conducted on 106 patients with chronic renal failure age range (20-69 years).

The patients were divided into two groups according to treatment, 40 patients with conservative treatment (12 Males and 29 Females) and 66 patients with hemodialysis treatment (44 Males and 22 Females). IL-6 were determined in serum of all patients by using a commercially available ELISA Micro wells kit from Diaclone France. Total with blood cells count and differential count in whole blood of all patients were determined by using an automated hematology autoanalyzers XT2000i (from sysmex, Japan).

Statistical Analysis

SPSS version 20 was used as Statistical analysis. The results comparison assessed by student t-test.

Results

The results illustrated total white blood cells count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils showed the normal range (4-10 x 10^9 / L, 2-7 x 10^9 / L, 1-3 x 10^9 / L, 0.2-1 x 10^9 / L, 0.0-0.5 x 10^9 / L and 0.0-0.1 x 10^9 / L) respectively in conservative group (7.7±0.3 x 10^9 / L, 4.8±0.3 x 10^9 / L, 1.9±0.1 x 10^9 / L, 0.5±0.0 x 10^9 / L, 0.2±0.0 x 10^9 / L, 0±0.0 x 10^9 / L) and in hemodialytic group (5.7±0.2 x 10^9 / L, 3.6±0.1 x 10^9 / L, 1.4±0.0 x 10^9 / L, 0.4±0.0 x 10^9 / L, 0.1±0.0 x 10^9 / L, 0.0±0.0 x 10^9 / L) respectively. However total white blood cells count, neutrophils, lymphocytes, monocytes, eosinophil and basophil decrease significantly (p<0.05) in hemodialytic group when compared with conservative group. IL-6 levels were in conservative group (16.4±3.4 pg/ml) and in hemodialytic group (26.0±5.96pg/ml), rise than the upper limit of the normal rang (5-15pg/ml), The IL-6 level was higher significantly (p<0.01) in hemodialytic group when compared with conservative group. As showed in table below.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Normal range</th>
<th>Conservative (n=42) Mean ± SE</th>
<th>Hemodialysis (n=66) Mean ± SE</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>5-15pg/ml</td>
<td>16.47±3.43</td>
<td>26.08±5.96</td>
<td>0.022S</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>4-10 x10^9/L</td>
<td>7.74±0.35</td>
<td>5.70±0.24</td>
<td>0.001S</td>
<td></td>
</tr>
<tr>
<td>NEU</td>
<td>2-7 x10^9/L</td>
<td>4.88±0.31</td>
<td>3.68±0.19</td>
<td>0.001S</td>
<td></td>
</tr>
<tr>
<td>LYM</td>
<td>1-3 x10^9/L</td>
<td>1.98±0.11</td>
<td>1.40±0.06</td>
<td>0.001S</td>
<td></td>
</tr>
<tr>
<td>MON</td>
<td>0.2-1 x10^9/L</td>
<td>0.57±0.03</td>
<td>0.42±0.02</td>
<td>0.001S</td>
<td></td>
</tr>
<tr>
<td>EOS</td>
<td>0.02-0.5 x10^9/L</td>
<td>0.26±0.02</td>
<td>0.18±0.02</td>
<td>0.029S</td>
<td></td>
</tr>
<tr>
<td>BAS</td>
<td>0.0-0.1 x10^9/L</td>
<td>0.03±0.00</td>
<td>0.01±0.00</td>
<td>0.001S</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Our current study shows white blood cells count and differential count decreased significantly in hemodialytic patients, but they were within normal ranges. That uremia related to immune dysfunction has been seen in some researches. However, abnormal immune dysfunction are recorded because of uremic toxins [12]. Prevalence of the studies focused on the abnormal dysfunction of WBC. Minnaqanti and Cunha pointed that subjects showed renal disorders impair defense of host [13]. Baqdasarian et al showed subjects with kidney disease on dialysis counter with infection which is a major cause of mortality and morbidity [14]. The studies revealed that there is a relationship between renal disorder and infection. Hayder et al., reported that total WBCs were decreased significantly at the ages 40-60 years of 111 CRF on dialysis, while total WBCs reduced statically in 50 CRF of HD patients [15]. Suresh et al., has been perceived that the total white blood cells are decreased in HD patients than the control. The specific mechanism by which chronic renal disease leads to a slight decrease in total white blood cells count is not clear. The possible hypothesis is as follows in chronic kidney failure patients undergoing dialysis, in the dialyzer, exposure of blood to artificial membranes may result in complement activation in vivo [16]. Agrawal et al., have recorded that antigen presentation dendritic cells decreased in uremia [17]. Decreasing number of B lymphocytes capacity for producing antibody have been recorded in uremic subjects by Pahl et al., [18]. Depleting of naïve and memory T cells in uremic cases has been recorded by Moser et al [19]. The total and differential count showed significant differences but values were within limit of the normal ranges that normal total and differential WBC count in one study was seen in majority of cases (28 cases 87.5%). Four cases (12.5%) revealed increased WBC count and neutrophilia, that the etiology of chronic renal failure was included type-II diabetes mellitus 8 cases (25%) obstructive uropathy 5 cases (15.62%), hypertension 3 cases (9.37%) and renal tuberculosis 1 case each (3.125%) and in remaining 14 cases etiology was not known [20]. While Agarwal and Light had reported that Patients with CKD had more eosinophils and granulocytes and less lymphocytes. Over time, granulocytes rise and lymphocytes reduced in those with and without CRD. In addition, in those with CRD, over time monocytes increased and eosinophils decreased [21].

IL-6 were as an immunological Parameters that cytokines are released in the course of HD session mainly by monocytes, and factors responsible for monocytes activation include endotoxins that may be present in dialysate fluid, activated complement and dialyzer membrane itself [22]. The physical interaction between artificial dialyzer membrane and some compounds in dialysate fluid leads to activation of alternative pathway of complement. The type of dialyzer membrane is critical in this course [23]. Our results of study showed serum concentrations of IL-6 are increased in hemodialysis patients. Several studies reported unchanged serum IL-6 during HD concurrent with increased clearance or membrane adsorption of these cytokines [24]. The reasons for this phenomenon maybe depend on cytokine kinetics. The half-life of IL-6 (3-7 min), are seem to be short..Plasma cytokines are quickly bound to cell surface receptors, this suggests that stable plasma concentrations are achieved by a continuously high production rate. As a consequence, the entire amount of cytokines potentially removed via hemodialysis is maybe significantly lower when compared with the endogenous production [25]. It is not unlikely that the serum concentrations of cytokines serve as target of feedback mechanisms, since their endocrine function has been displayed, apart from paracrine functions [26]. Thinking that adsorption of cytokines happens mainly in the first minutes of HD and may not reflect a substantial amount of cytokine elimination [25]. HD membranes may rise cytokine production by activating mononuclear cells [27].

Conclusion - hemodialysis session impaired immune system and activation anti-inflammatory factors.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad AlRafidain University College and all experiments were carried out in accordance with approved guidelines.
References


Medical Induction of First Trimester abortion by Misoprostol or Misoprostol with Letrozole

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Abstract

Background: Managing of abortion is an important subject in gynecology. Millions of patients had suffered from spontaneous abortions that occur per year.

Objectives: The aim of this study was to compare the effect of misoprostol alone and misoprostol with letrozole in the induction of abortion in the first trimester of pregnancy.

Patients and Methods: In a single –blind clinical trial, 128 female applicants for legal abortion within the first trimester of pregnancy that referred to AL-Batool hospital, were divided in to two groups: intervention (misoprostol with letrozole) and control groups (misoprostol alone). The complete abortion, drug-induced side effects of misoprostol and letazole were noted and evaluated.

Results: The complete abortion rate was 93.2% in intervention group and in 68.7% in the control group (misoprostol) only, which was significantly higher in intervention group (p = 0.001).

Abdominal pain in the intervention group is also significantly lower than that of the control group (p = 0.013). Intervention group also had significantly lower duration of bleeding rather than control group (p = 0.006).

Conclusions: Based on our results, pretreatment with letrozole plus Misoprostol was more effective for prompting abortion in the first trimester of pregnancy without increasing side effect compared to misoprostol alone.

Keywords: Letrozole; Abortion, Induced; Misoprostol, Pregnancy, First rimester;

Introduction

Regarding the national center of vital statistics, centers for disease control and prevention and the world health organization definition, abortion is the termination of a pregnancy before the 20th week of pregnancy or termination of pregnancy before the fetus weighing 500 g (¹, ²).

Abortion managing is an important subject in gynecology. Millions of spontaneous abortions happen, and more than a million of induced abortions are occur yearly either by drugs or surgical techniques. Definitive treatment for abortion is a surgical practice yet it is invasive and costly, is not required for all females.

According to the American college of obstetricians and gynecologists (2005), medical abortion is an suitable alternative for surgical techniques in pregnant women with gestational age of less than 49days founded on the last menstrual period (LMP) (³).Spontaneous abortion happens in 10% - 20% of whole pregnancies, while, 80% of abortions occur earlier the 12th week of pregnancy. (⁴)

In medical therapy, abortion done by various drugs, considering mifepristone drugs has limited access, its high cost, it is not available in most of the countries and alternative drugs are used to induce abortion (⁵). One
of these drugs which used both vaginally and orally is prostaglandin E1 analogue, misoprostol, which is Known as Cytotec trade mark. Misoprostol is a cheap drug which could be kept in room temperature and usually used as vaginally and orally \((6)\). Misoprostol is widely used for induce of labor, softening of the cervix, curettage, hysteroscopy, therapeutic abortion, endometrial biopsy, early termination of pregnancy, treatment of incomplete abortion or missed abortion and treatment of postpartum hemorrhage \((3)\). Use of 800 mcg of vaginal misoprostol results in abortion by exciting the myometrium \((7)\). Oral or vaginal rout of administration of misoprostol cause complete abortion in nearly 85% of cases within seven days before the 12th week \((6,7)\).

Letrozole is a non-steroidal aromatase inhibitor for the treatment of estrogen-dependent breast cancer. Estrogens are required for continuation of the pregnancy. Letrozole is competitive reversibly bonds with cytochrome P450 and inhibits the production of estrogen by the enzyme aromatase. Aromatase inhibitors are commonly used to treat patients with breast cancer \((8-9)\).

Aromatase inhibitors have no androgenic, progesterone and estrogenic effects. It seems that aromatase inhibitors, such as letrozole, can open a new path and be a significant therapeutic option in the treatment of females. Letrozole is an aromatase inhibitor that can amplify the effect of misoprostol in first trimester abortions. Aromatase is an enzyme that is secreted from the placenta, ovarian granulosa cells and other tissues, such as fat, muscle, brain and breast tissue. Letrozole has been used in the treatment of estrogen-dependent breast cancers \((7)\).

**Materials and Methods**

In a single-blind randomized, A 128 women were included in our study, study protocol was approved by the Ethics Committee of Diayla university, Diayla medical college, during June 2016 to April 2018, Considered as two groups of intervention and control after consent of patient and her spouse to participate in the study, who age above 18 years old were referred to AL Batool Teaching hospital .

Inclusion criteria were involved:

1. Age of patient over 18 years old.
2. Gestational age less than 64 days gestation, missed abortion. (<9 wks).
3. Hemoglobin >10 g/dL.
4. Absence of any maternal illnesses such as: heart disease, asthma, past history of thromboembolism, cancer, renal failure, and liver illnesses.

Where exclusion criteria as followed: any medical problematic in patient which necessity to interfere and emergency management, history of allergy to misoprostol or letrozole drugs or Coagulopathy problem.

A 128 patient divided into two equal group, Sixty-four patients in intervention group and 64 patients in control group. In intervention group to encourage drug abortion, patients first received 10 mg letrozole per day orally for 3 days, then they received 600 microgram oral misoprostol as single dose. While in control group patients first daily established placebo of letrozole similar intervention group and then 600 microgram single dose oral misoprostol was used. In both groups, patient who had spontaneous abortion before starting treatment of misoprostol in the first three days of study, were excluded from the study.

Patients in both groups hemoglobin levels were examined at the beginning and end of the study. Serum estradiol levels were measured at the beginning in control group and measured at the beginning and before taking misoprostol in intervention group.

Both groups were checked for 4 hours after receiving single dose of misoprostol and were observed for probable side effects such as abdominal cramp or possible bleeding, and in terms of absence of abdominal cramp or severe bleeding, were released after explanation risk and threatening signs such as bleeding more than normal menstruation.

Subsequent visit and monitoring of patients in both groups was showed 15 days later and response to treatment and complete abortion, amount and period of bleeding, drug side effects such as nausea, vomiting, diarrhea, headache, dizziness, pain in the lower parts of the abdomen, and ague all were detected and ultrasound to perform in both groups to check uterine cavity and measuring the hemoglobin level. If remnants of pregnancy or failure in termination of pregnancy,
repeated doses of misoprostol or curettage can be done. In both groups of control and intervention were put in two categories of response to treatment and failure in response to treatment.

Statistical analysis was done by SPSS, software package version 16.0 for windows (SPSS Quantitative data were accessible as mean ± standard deviation (SD), while qualitative data were established as frequency and percent (%). To compare qualitative variables chisquare statistical test was used and to relate quantitative variables between two considered groups independent T-test was implemented. Also to associate intra-group quantitative variables such as hemoglobin, paired T-test was applied. Significance level was considered as p < 0.05.

### Results

<table>
<thead>
<tr>
<th>Intervention group (letrozole group)</th>
<th>Control group P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age of patients (N=)</td>
<td></td>
</tr>
<tr>
<td>29 ± 4.08 years</td>
<td>28.53±5.25 years</td>
</tr>
<tr>
<td>Mean pregnancy age (N=)</td>
<td></td>
</tr>
<tr>
<td>7.74 ± 0.95 weeks</td>
<td>8.52 ± 1.29</td>
</tr>
<tr>
<td>BMI (KG\M2) (N=)</td>
<td></td>
</tr>
<tr>
<td>23.9±5.0</td>
<td>22.8±5.0</td>
</tr>
</tbody>
</table>

Table 1: Demographic data shows, women age, body mass index and gestational age of pregnancy in both groups.

No significant difference were found in the women age, body mass index and gestational age of pregnancy.

<table>
<thead>
<tr>
<th>Intervention group Letrozole + Misoprostol</th>
<th>Control group Placebo + Misoprostol</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Intervention</td>
<td>13.29±0.95</td>
<td>13.02±0.86</td>
</tr>
<tr>
<td>After Intervention</td>
<td>12.55±1.01</td>
<td>12.14±0.97</td>
</tr>
</tbody>
</table>

Table 2: Hemoglobin level (mg/dl) in both Intervention and Control groups

Decrease of Hemoglobin level in the intervention group was statistically significantly lower than control group.

### Table 3. Complete abortion in both groups

<table>
<thead>
<tr>
<th>Intervention group Letrozole + Misoprostol</th>
<th>Control group Placebo + Misoprostol</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complet abortion (%)</td>
<td>93.75%( n=64)</td>
<td>68.55%(n=44)</td>
</tr>
<tr>
<td>Surgical interference (%)</td>
<td>6.25%(n=4)</td>
<td>31.25%( n=20)</td>
</tr>
</tbody>
</table>

In terms of response to treatment, two groups had statistically significant difference (p = 0.001)

And need for surgical curettage in intervention group was significantly lower than that of control group (p = 0.012).
Table 4. Complications in the period of study in individually in Intervention group and Control group

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Letrozole + Misoprostol</td>
<td>Placebo + Misoprostol</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>59.3%</td>
<td>70.3%</td>
<td>0.218</td>
</tr>
<tr>
<td>Vomiting</td>
<td>29.6%</td>
<td>26.6%</td>
<td>0.710</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>31.2%</td>
<td>43.8%</td>
<td>0.113</td>
</tr>
<tr>
<td>Dizziness</td>
<td>28.1%</td>
<td>25.0%</td>
<td>0.848</td>
</tr>
<tr>
<td>Headache</td>
<td>6.2%</td>
<td>4.7%</td>
<td>0.728</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>54.6%</td>
<td>76.6%</td>
<td>0.013</td>
</tr>
<tr>
<td>Fever</td>
<td>6.2%</td>
<td>1.6%</td>
<td>0.127</td>
</tr>
<tr>
<td>Chills</td>
<td>10.9%</td>
<td>9.4%</td>
<td>0.786</td>
</tr>
</tbody>
</table>

Drugs side effects in two studied groups during the study, have been obtainable in table 3. the abdominal pain in interventional group was significantly lower than that of control group. (P=0.013).

Table 5: Bleeding duration in both interventional and Control groups

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Letrozole + Misoprostol</td>
<td>Placebo + Misoprostol</td>
<td></td>
</tr>
<tr>
<td>Within a few hours</td>
<td>0.00%</td>
<td>7.81%</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Within a day</td>
<td>60.93%</td>
<td>42.19%</td>
<td>P=0.001</td>
</tr>
<tr>
<td>More than a day</td>
<td>39.06%</td>
<td>50.00%</td>
<td>P=0.001</td>
</tr>
</tbody>
</table>

Generally comparing both groups in expressions of duration of bleeding, intervention group had significantly lower duration of bleeding rather than control group (p = 0.006)

Discussion

Medical abortion reduce side effects such as bleeding and infection, and also stress in patients, relating with surgery. In medical abortion technique it is possible to use several drug regimens to induce abortion. Misoprostol is a prostaglandin analog and a benign and cheap drug which is used for inducing medical abortion and frequent studies have shown its efficiency\(^{10, 11}\).

In a pilot study, in persuading abortion in 40 legal below r-63-day abortion cases, 7.5 mg letrozole for 2 days, and then usage of vaginal misoprostol leads to preform abortion in 80% of patients and comparing with Mifepristone, using of letrozole with misoprostol together was more efficient than combination of letrozole and mifepristone\(^{12}\). In the present study also success rate of complete abortion was higher in using misoprostol and letrozole comparing with using misoprostol alone (p = 0.001).\(^{11, 12}\)
In a prospective study success rate in prompting abortion for 56-day or less pregnancies using single dose misoprostol vaginally, was informed 72%. Also this study claimed that in case of failure in access to mifepristone, using misoprostol only clinically could lead to suitable outcomes (13). Also a evaluation of this study has revealed that in treatment of missed abortion in the first trimester of pregnancy by misoprostol, 800 microgram misoprostol as single dose per vagina or 600 microgram single dose sublingual misoprostol are good substitutes for surgery. This study also expressed that after advising misoprostol there was no necessity for hospitalization and patients would refer in case of severe bleeding or infection (14). In the current study also frequency of complete misoprostol for using misoprostol alone was 68.75%, and in case of lack of admission to another drug, using this drug had suitable outcomes similar other studies (13,14).

In a pilot study, up to the ninth week of pregnancy in 50 person, a single dose of 200 mg mifepristone and 10 mg letrozole per day for 3 days, and lastly 800 microgram single dose 1 misoprostol per vagina, were used to persuade abortion rate of complete abortion in this study was 98%, and also no significant side effects were informed (15).

Similar to recent study, in a clinical trial, on 168 women below -63-day pregnancy age, letrozole treatment before misoprostol was examined. Success rate in complete abortion in letrozole getting group was significantly higher than that of group getting misoprostol alone. Between side effects, vomiting was significantly more in letrozole group than in control group (16). In current study using letrozole did not lead to rise in side effects and even abdominal pain and bleeding time in intervention group was significantly lower than that of control group. Also abdominal pain in letrozole receiving group was lower than that of control group. Finally numerous new studies express that, implementing effective drug regimens for inducing abortion is more affordable and risk free than methods such as vacuum aspiration (19, 20), which should be paid attention by physicians.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of anesthesia Bilad AL Rafidein University College and all experiments were carried out in accordance with approved guidelines.

References
Study Some Haematological And Biochemical Parameters in Patients with Renal Failure in Diyala Province

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Abstract

Current study was happened in Bâqubah province for kidney failure patients (in Ibn Sina Center) for dialysis in Bâqubah teaching hospital and started from September (2016) to March (2017). (100) samples of blood collected from patients of renal failure, males number (61) and females (39) with age range (10–88) years and collected (50) samples of blood from healthy individual have been accredited as group control, males number (25) and females (25) with age range (10–88) years. Result of current study appeared that the ratio of disease rate among males (61%) than females (39%) with no significant different between groups (P>0.05). Result of biochemical parameters expressed increased levels of B.urea, and S.creatinine For patients when compared with control with significant different between groups (P<0.05), so uric acid was raised For patients when compared with control but with no significance different between groups (P>0.05), either albumin and total protein parameters were lowest For patients when compared with control with significance different between groups (P<0.05). The hematological parameters (HB, PCV, WBC and PLT) result were noticed decreased For patients when compared with control with significance different between groups (P<0.05). The conclusions of our study showed the percentage of male patients are more than female, age periods (31-50 and 51-70) years have high percentage of patients, low levels of all hematological parameters (HB, PCV, PLT and WBCs) in patients than controls, high levels of biochemical parameters (B.urea, S.creatinine, Uric acid) in patients than controlsm, while (Albumin and protein) were low in patients than controls. The aim of current study is detecting hematological and biochemical parameters changes in patients with Renal failure in Bâqubah province.

Key words: renal failure, biochemical parameters, hematological parameters.

Introduction

Acute renal failure appear a rapid losing in renal function leading to kidney function loss resulting and increased nitrogenous waste in blood and fluid balance. Is a common threat to people with serious illnesses in intensive care units, in addition to the mortality rate of 42% to 88% [1].

Renal failure is the kidney’s inability to remove metabolic end products from blood and dysregulation on fluid, electrolytes, and pH balance of fluids. It may be the cause of kidney disease, urinary tract defects of a non-ethnic origin, or systemic diseases and kidney failure is divided into acute or chronic. Acute renal failure is reversible damage of kidney that may be treated [1][2].

Millions People are affected by non-fatal kidney disease, urinary tract infection, blockage and kidney stones are considered to be the most important diseases observed in the kidneys. Up to 20% of all women have urinary tract infections at some time in their lives and at least 1% of patients develop kidney stones [3]. Chronic kidney disease (CKD) is a syndrome characterized by gradual and irreversible deterioration of kidney function due to the slow destruction of the kidney weft and consists of a wide range of different physiological pathological processes, which are associated with chronic renal failure, and eventually ends in death when adequate numbers of the Nephrons. Acidosis is the main problem in CRF with the development of biochemical asymptomatic and clinical uremia syndrome. Normal
renal function and progressive reduction of glomerular filtration rate (GFR)\textsuperscript{4,5}

Ki In kidney disease is associated with many hematological changes. Anemia is similar to the degree of renal insufficiency and the most important reason is the failure of renal erythropoietin secretion. In addition to chronic blood loss, blood dissolution and bone marrow fracture through retained uremic factors\textsuperscript{6}

The aim of current study is detecting hematological and biochemical parameters changes in patients with Renal failure in Baqubah province.

**Material and Methods**

Current study was happened in Baqubah province for kidney failure patients (In Ibn Sina Centr) for dialysis in Baqubah teaching hospital and started from September (2016) to march (2017). (100) samples of blood collected from patients of renal failure, males number (61) and females (39) with age range (10–88) years and collected (50) samples of blood from healthy individuals have been accredited as group control , males number (25) and females (25) with age range (10–88) years. Collected 5 ml of blood and it was left at room temperature 20-25°C to allow it to clot, then the sera was separated by centrifugation for (5-10) minutes, and sera divided into three tubes (250 µl) and stored at -20°C still examination.

**Parameters of current study**

1- Hematological parameters

The hematological parameters (HB, PCV, PLTs and WBCs) were measured by Complete Blood Count (CBC) system.

2- Biochemical parameters

The Biochemical parameters (B. urea, S. creatinine, Uric acid. Albumin and protein) were measured by Cobas 400.

**Statistical Analysis**

Chi-squared and T test are method used to analysis of current data, a level of significance of P<0.05 was applied to test, the statistics software used to process the data analysis were the (SPSS v.22)\textsuperscript{7}.

**Results and Discussion**

Results of current study showed the percentage of males more than females with non-significant difference between sex as well as two groups p>0.05 and shown as in table (1).

| Table (1) Distribution of study groups according to sex. |
|----------------------------------|---|---|
| **Study group** | **Control** | **Patients** |
| Male | 25 | 61 |
| % | 50 % | 61 % |
| Female | 25 | 39 |
| % | 50 % | 39 % |
| Total | 50 | 100 |
| p value | No Sig. (p>0.05) |
Results of current study show that the percentage of males with renal failure more than females and reasons of it may be due largely to the daily effort after exposed then women, large number of protein consume by men than women, in addition to that muscle mass in men than women leads to increase concentration of creatinine in men \[8\]. In addition, result of current study were compatible with results conducted by \[9\].

In current study the age range of renal failure patients were 10-88 years as shown in table (2). Also the current study results showed that the age periods 10-30 and >70 years recorded lowest rate of disease, while the age period 31-50 and 51-70 years recorded highest rate of disease. The age of control group range between 10-88 year and divided into groups, and found significant different between groups P<0.05 as shown in table (2).

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Study group</th>
<th>Control</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-30 years</td>
<td>15</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>30%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>31 -50 years</td>
<td>15</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>30%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>51-70 years</td>
<td>15</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>30%</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>10%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Moreover, results of current study were agree with results that conducted by \[10\]. Chronic kidney diseases (CKD) are commonly in elderly \[11\] and this is why the professional organizations to conduct routine tests for older people in health care centre \[12\]. The individuals have >55 year are more susptible to renal failure disease as compared to young \[13\]. The age group associate with glomerular filtration rate (GFR), the elderly groups have low (GFR) than young groups \[14\]. So it will be that, percentage of death for patients with kidney failure patients elderly more than young \[15\].

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In current study we measured the haematological parameters in patients and control. Notice decrease all haematological parameters in patients than control, where the concentration of HB and PCV were low in patients (9.38±1.85 g/dl and 28.54±5.30 %) than control (13±0.69 g/dl and 44.78 ± 5.9% ) respectively with high different significant (P<0.05). Also, the current study show decrease concentration of WBC and PLT in patients (6120±2663.93 cell/ml and 211.23±90.86 cell/ml) than control (7607.72±2557.43 cell/ml and 278.7 ±99.24 cell / ml) respectively with high different significant (P<0.05) as shown in table (3).
Table (3) Comparison haematological parameters between study groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number</th>
<th>Mean</th>
<th>S.D</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB g/dl</td>
<td>patient</td>
<td>100</td>
<td>9.38</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>50</td>
<td>13</td>
<td>0.69</td>
</tr>
<tr>
<td>PCV %</td>
<td>patient</td>
<td>100</td>
<td>28.54</td>
<td>5.30</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>50</td>
<td>44.78</td>
<td>5.9</td>
</tr>
<tr>
<td>WBC cell / ml</td>
<td>patient</td>
<td>100</td>
<td>6120</td>
<td>2663.93</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>50</td>
<td>7607.72</td>
<td>2557.43</td>
</tr>
<tr>
<td>PLT cell / ml</td>
<td>patient</td>
<td>100</td>
<td>211.23</td>
<td>90.86</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>50</td>
<td>278.7</td>
<td>99.24</td>
</tr>
</tbody>
</table>

In current study WBCs was low in patients than controls and this low was agree with study [21]. Low causes of WBCs in patients with renal failure includes:-

1- Leukocytopenia is related to inflammation.
2- High concentration of pro-inflammatory cytokines and specially TNF-α which lead to inhibition leukocytosis [21].

In addition, PLTs levels were low in current study and our study was agree with study [22] that refer two decreases levels of PLTs in patients with renal failure.

Platelets circulate in the blood of mammals and participate in blood clotting which leads to the formation of blood clots. Thrombocytopenia plays a major role in blood clot events, especially in the identification of heart disease and atherosclerosis in patients with end-stage renal failure [23].

In current study we measured the biochemical parameters in patients and control. Notice increase some biochemical parameters (B. sugar, B. Urea, Creatinine , Uric acid) in patients than control, and decrease protein, Albumin . The concentrations of B. Urea were high in patients (102.65 ± 42.98 mg /dl ) than to control (31.48±9.73 mg /dl) respectively with high different significant (P<0.05). so The concentrations of Creatinine was high in patients (5.75±2.09 mg /dl) than to control (5.31 ± 1.64 mg /dl) respectively with no different significant (P>0.05), while the protein and Albumin were low in patients (63.62±8.71 g /dl and 36.16±6.28 g /dl) than to control (70.92±5.92 g /dl and 44.52 g /dl ± 6.36 g /dl) with high different significant (P<0.05). as shown in table (4).
This study is agreed with the researches [24] which are includes high levels (Uric acid and S.urea) in patients with renal failure when compared with control group. the hemodialysis which diabetic dialysis patients ,they are more likely to develop cardiovascular disease compared with non-diabetic dialysis patients.

The results show that an increase urea level in in serum blood for patient with hemodialysis when compared with control group ,may be due to the urea is the first organic solute detected in the blood of Patients with renal failure had the most significant quantified melanin secreted by kidneys in patients. However, however, we need many research showing signs and symptoms of uremic recurrence by raising levels of soluble in patients with polina [25].

On other side result of study Urinary symptoms in patients were found to be alleviated by initiation of renal dialysis, even when urea was added to the aura to maintain the level of nitrogen in the blood at about 90 mg per deciliter [26].

The result show high level in serum creatinine for patient with renal failure when compared with control group. creatinin is an excreting substance that is produced in creatin metabolism in a nonenzymatic pathway. High level in creatinine and urea may be due to pre renal or post renal disorders [27].

Our study had several important limitations. Glomerular filtration rate (GFR)is of dises which are based on serum creatinine however, they can potentially lead to over diagnosis of chronic kidney disease [28].

In dialysis patients low serum creatinine is a marker for protein-energy wasting, which is a strong predictor of anemia and mortality in renal transplant recipients [29]. A diagnostic test to assess kidney function is used to Determination of serum creatinine. [30] show The doctors rely on plasma concentrations of waste materials from creatinine and urea. These tests are sufficient to determine whether a patient is suffering from kidney disease and help to measure the efficiency of the kidneys in filtering the blood. It also gets the amount of nitrogen and creatinine in the blood increase.

GFR is used to show how determine the stage of renal disease and guide decisions about treatment and the renal function of the patient still has. [31] Renal renal failure is caused by conditions that damage structures within the kidney - glomerular, interstitial or tubular. More common injury than common tubes is toxic in origin or ischemia. That ischemia associated with clinical failure and obstruction within the nucleus. The toxic abuse of tubular structures, acute glomerulonephritis,
acute erythema and kidney disease are the major causes of kidney failure [31].

On other hand high level in serum uric acid may be to development of renal dysfunction, a potential mechanism by U.A. Kidney disease may be exacerbated by activation of the renin angiotensin system (RAS). Research has found that RAS plays a role in worsening kidney disease by increasing the level of glomerular and systemic pressure, in addition to its direct cause of cirrhosis and damage to blood vessels and renal cells. In the early stages of hypertension that causes an increase in U.A leads to a decrease in renal blood flow which in turn causes a change in the balance between the turnover of the spinal cord and the cortical cycle, which may lead to a decrease in urate secretion [23].

A new approach toward the prophylaxis and treatment of uric acid nephropathy is the enzyme uricase, which catalyze station of uric acid causing mechanical obstruction, direct toxicity to endothelial cells and epithelial, and potentially activation of the innate immune system [27].

In this study the results show decreased signification in albumin level in patients with renal failure. This result is a gree with [34] and [33]. The effects of nutritional status and inflammatory association with the number of deaths in dialysis patients [20] and some studies show albumin level relationship increased 47% in the risk of death. The increase in death risk was partially explained by the inflammatory pathway. The albumin is negative in the acute negative phase, and the level of albumin in the serum is strongly affected by the presence of inflammatory, serum albumin measurements in patients with dialysis patients can not accurately assess dialysis patients [34] show Decrease in serum albumin levels may be associated with increased blood viscosity and hypercoagulable states [28]. The albumin plays an important role as a free radical activator, a carrier of a wide range of drugs, hormone and binding agent for toxic compounds. The mucosal pressure may negatively affect fluid transfer between interstitial space and intracellular vacuum. [29]. On other hand the results show the total serum protein decreased signification in patients renal failure when compared with control group ,the concentration in total protein because of ultrafiltration, which leads to is low protein binding which tend to decrease further during dialysis and peculiar behavior of hip uric acid [30][31].

Conclusions

The conclusions of our study showed:-

1- The percentage of male patients are more than female.

2- Age periods (31-50 and 51-70) years have high percentage of patients.

3- Low levels of all hematological parameters (HB, PCV, PLT and WBCs) For patients when compared with control.

4- High levels of biochemical parameters (B. urea, S.creatinine, Uric acid) For patients when compared with control , while (Albumin and protein) were low For patients when compared with control.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad Alrafidain University College and all experiments were carried out in accordance with approved guidelines.

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Thyroid Carcinoma Patients Correlated with Cytomegalovirus by using Insitu Hybridization

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Abstract

Worldwide, the most common cancer-related deaths and poses one of the top ten most risk cancers globally in many countries, is the thyroid carcinoma. In human cancers, there are important factor associated with it. The viral agent is one of them which is involved in the spread of tumors, including thyroid cancers. So, the present study was designed to determine the relationship between Cytomegalovirus infection with some Iraqi patients who suffer from the thyroid carcinoma in tissue cancer from different site (papillary, follicular and lymph node) by using In Situ Hybridization Technique, related to their age, gender, site distribution, histological grade and the tumor stage. The sixty biopsies were randomly collected during the period between June 2017 until October 2018, CMV was detected in (28.3%) (17 out of 60 patients, the mean age was 39 years old, ranged between (27-72) years old, female to male ratio was (1.3 : 1) with 34 women and 26 men. The most of these cases were papillary carcinoma 39, followed by follicular 13 and the rest 8 cases were at the lymph node. Histological grades that involved were 37 well differentiated, 18 were poorly differentiated, and 5 were moderately differentiated. Tumor staging included 43 patients who fall in stage I and II, while the rest 17 patients fall in stage III and IV. Regarding to the positive results, CMV correlates in a highly significant association with each of (age, gender, grading, tumor stage, and site distribution at p<0.01).

It could be concluded from that the in situ hybridization is useful in the clinical evaluation of patients with thyroid carcinoma. And suggested that the viral agent cytomegalovirus is involved in thyroid carcinogenesis and may play an important role in the malignant transformation of thyroid cancer.

Keyword: Thyroid cancer, cytomegalovirus, Insitu hybridization.

Introduction

Thyroid cancer is the most common cancer in the head and neck and the major tumor malignant of the endocrine gland(1). thyroid cancer types which including the well differentiated papillary (80% of the cases), and follicular (15% of the cases),while the latter being divided into conventional and oncocytic type (Hürthle cell), anaplastic carcinoma as well as poorly differentiated carcinoma(2), poorly differentiated and anaplastic carcinoma are both can arise de novo, or secondarily from papillary thyroid cancer and follicular thyroid cancer (3).

The early stage well differentiated papillary patients as well as the follicular carcinoma patients usually have an excellent prognosis, whereas, the patients with either aggressive tumors or distant metastases, have a 5-years survival rate of 40% (4). Unlike the thyroid cancer three aforementioned types, medullary cancer, which derives from the neural crest, more specifically, parafollicular, or C cells (5). It accounts for about 3–4% of all cases of thyroid cancer(6), its clinical course varies from the indolent to rather aggressive, with high mortality rates association (5). Primary thyroid lymphoma represents the rare non-Hodgkin lymphoma (7) which derives predominantly from the B-lymphocytes (8).

From 2000 until 2014, the thyroid cancer incidence for both individuals of both genders has significantly increased, particularly for females aged between 55 to 64 years (9). The increase rates refers to tumors in all stages. The reasons for this increase have not been completely
Many risk factors associated with thyroid cancers, for instance, viral agents are considered as a carcinogenic risk factor in more than 20% of human cancers \cite{10,11,12}. Human Cytomegalovirus have been implicated in the etiology of many human malignancies like breast cancers, cervical cancers, prostate cancers, colon cancer and thyroid cancer \cite{13,14}.

Many molecular methods can be used to identify the nucleic acid of viruses like HCMV, the one of these is InSitu Hybridization (ISH) method which can be used the frozen tissues and preparation of cytology as well as the tissue fixing by using radioactive labeled probes and also with non-radioactive labels as fluorescent moieties, biotin and enzyme conjugated probe \cite{15}.

**Materials and Methods**

This study was designed as a retrospective study, involved thyroid tissue samples which were collected as archival tissue blocks, during the period from June 2017 until October 2018, the series of sixty formalin fixed paraffin embedded tissue samples which were enrolled in the current study had been biopsied with patients data age, gender and diagnosis of histopathology and the site distribution. sixty patients with thyroid cancer (mean age 39 years) ranged between (27-72) years, female to male ratio 1.3 :1 with 34 women and 26 men. They have collected randomly from Teaching laboratories/ Medical city in Baghdad. Whom already diagnosed as a (papillary, follicular and lymph node ) of thyroid cancer by a specialist, They are compared with 10 healthy control where their data of ages and sex were matched to patients group.

preparation technique of the slides and tissue sectioning was done in the teaching laboratories/ Department of Histology/ Medical City/ Baghdad. The first tissue section mounted in ordinary slide to be stained with Hematoxylin and eosin, and the two subsequent tissue sections were mounted in the charged slides to be used for In Situ Hybridization for detection of DNA of the HCMV. In Situ Hybridization technique for molecular detection of CMV DNA in these tissues were performed by high sensitivity generation ISH as steps follow:

1- Pre-hybridization step : All the samples were deparaffinized and dewaxed by Xylene and with series of ethanol 100, 90 and 70% then in D.W. after that immersing in citrate buffer (pH: 6), deproteinization by placing in the proteins K solution, then dehydration by immersing these slides in D.W. and 70, 90 and 100% ethanol.

2- Hybridization step: By adding CMV probe to each slide, and placing them in an oven at 98 C° to denaturation of the DNA probe, then removing and incubation for overnight at room temperature to allow hybridization of the probe with the target nucleic acid.

3- Post hybridization step: By using protein block buffer to falling off all coverslips then conjugating onto all sections, using substrate, and counterstained with ethanol, xylene and mounted with DPX.

**Statistical Analysis**

The statistical analysis system – SAS \cite{16} was used to effect of differences factors in study parameters. The chi-square $\chi^2$ test at the comparative between percentage in this study.

**Results and Discussion**

The differentiated thyroid carcinoma arising from a follicular epithelial is the commonest endocrine malignancy, while papillary thyroid carcinoma accounts for the majority of the differentiated thyroid cancer \cite{17}. That given the fact in which the prevalence of non-medullary familial thyroid cancer in about only 5% \cite{18}, differentiated thyroid carcinoma is mostly sporadic and the only established epidemiological factor is associated with thyroid cancer are ionizing radiation as well as iodine deficiency \cite{19}.

The sixty paraffin embedded tissue blocks of thyroid carcinoma patients were enrolled and studied in the current study by using In Situ Hybridization method for detection of Cytomegalovirus infection and as shown in table(1). Cytomegalovirus detected in 17 out of 60 (28.3%)of patients and this disagreed with other finding research \cite{20} who reported no relation between CMV and thyroid cancer by using PCR technique for CMV DNA. There is no longer research for thyroid cancer with HCMV in Iraq. But the virus cytomegalo is detected in many cancers type by using the InSitu hybridization
method like with head and neck OSCC in (36.6 %) of patients (21). Mean age of the patients was (39 years old), 38 out of 60 were under 40 years of age and 22 were above 40 years (figure 1) and there is a highly significant correlation between CMV and the age of patients at P<0.01. Some previous studies have shown that thyroid cancer percentage was noticed to increase by age, and the likely recurrence of cancer is lower in young patients. Also besides, age played a significant role in the survival disparity, and the mortality rate is starting to climb at age of 45, and the rate of recurrence starting at age 60 years (22). The mortality risk was 5.4 times higher in aged >45 years versus <45 years (23), and the presence of distant metastases, the age remains as a strong prognostic indicator (24). It could be suggested there is a correlation between age and cancer development, because the prolonged exposure to lots of factors that may promote cancers, same as viruses and radiation and some chemicals (25). According to the thyroid carcinoma site distribution, the most affected site was the papillary 39 out of 60 (65%), followed by the follicular 13 out of 60 (21.6%) then the lymph node 8 out of 60 (13.3%) as in (figure 2). Statically there is a significant association between CMV and the site distribution at P<0.01. The present study, in agreement with (26) who found that papillary carcinoma accounts for 71% of the total thyroid malignancies in that studied. Even though confirmed with (27) and (28) who also observed the incidence of papillary carcinoma to be 76.6% and 78.56%, respectively. The major type of malignant tumor among all thyroid carcinomas worldwide, comprising an estimated 80% of thyroid cancers (29). As in (Figure 3) shows the female: male ratio is 1.3:1 and the gender distribution is 1.3:1 with 34 females and 26 male and statically there is a significant association at P<0.01. Our findings confirmed with other studies (26,30) which observed that female predominance in their studies with the female: male ratio of 5:1 and 2.6:1, respectively. It suggested that thyroid cancer and thyroid disease have more incidence rates in females than in male, because the females is more exposure to hormonal changes than males. Regarding the grade of the tumor and as shown in (figure 4), the well differentiated is the most predominant type, it was 37 out of 60 cases followed by poorly differentiated 18, then 5 cases were moderately differentiated with the significantly statistic association at P<0.01. In many previous studies of thyroid cancer related viruses the major samples were well differentiated because the main type which derives from follicular epithelial cells is well-differentiated papillary followed by well differentiated follicular carcinoma then the poorly and moderately differentiated. (3,30,31). In (figure 5) the predominant stage of the tumor is I, II 43 out of 60, whereas, 17 out of 60 were fell in stage III, IV, statically there is a significant correlation at P<0.01. This finding was disagreed with (20) who found and reported that is More than one-third of their patients fell in stage III and IV and thyroiditis did not correlate with tumor stage.

In our study, it recognized some of the limitations, the patients number that studied was small, So as the results obtained from selected population maybe not extrapolated to the other population. Some previous study has shown that CMV could widely distribute in other organs (21,32).

<table>
<thead>
<tr>
<th>Table 1: (Distribution of patients with thyroid cancer according to their age, gender, histological grade, site distribution and tumor stage correlation with Cytomegalovirus by using In Situ Hybridization technique)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors</strong></td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>27-40</td>
</tr>
<tr>
<td>41-72</td>
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</tbody>
</table>
Table 1: (Distribution of patients with thyroid cancer according to their age, gender, histological grade, site distribution and tumor stage correlation with Cytomegalovirus by using In Situ Hybridization technique)

<table>
<thead>
<tr>
<th></th>
<th>Grade</th>
<th>Chi-square (P-value)</th>
<th>Stage</th>
<th>Chi-square (P-value)</th>
<th>Site</th>
<th>Chi-square (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.224 NS (0.166)</td>
<td></td>
<td>9.67 ** (0.001)</td>
<td>Papillary</td>
<td>2.166 NS (0.108)</td>
</tr>
<tr>
<td>Grade</td>
<td>Well</td>
<td>25 (67.57%)</td>
<td>I</td>
<td>30 (69.76%)</td>
<td>28 (71.80%)</td>
<td>2.166 NS (0.108)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2 (40%)</td>
<td>II</td>
<td>13 (30.24%)</td>
<td>11 (28.20%)</td>
<td>2.166 NS (0.108)</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>16 (88.89%)</td>
<td>III-IV</td>
<td>13 (76.47%)</td>
<td>9 (69.23%)</td>
<td>1.666 NS (0.274)</td>
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<td></td>
<td></td>
<td>12 (32.43%)</td>
<td></td>
<td>4 (23.53%)</td>
<td>4 (30.77%)</td>
<td>1.666 NS (0.274)</td>
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<td></td>
<td></td>
<td>6 (75%)</td>
<td>2.166 NS (0.108)</td>
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<td>2 (25%)</td>
<td>2.166 NS (0.108)</td>
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<tr>
<td></td>
<td></td>
<td>2.055 NS (0.274)</td>
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<td>2.055 NS (0.274)</td>
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<td></td>
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<td></td>
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<td></td>
<td>Papillary</td>
<td>2.166 NS (0.108)</td>
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<td></td>
<td></td>
<td>Follicular</td>
<td>2.166 NS (0.108)</td>
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<td></td>
<td>Lymphnode</td>
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<td>2.055 NS (0.274)</td>
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</table>

** (P<0.01), NS: Non-significant.

Figure 1: Distribution of thyroid cancer patients according to their age

Figure 2: Thyroid cancer patients according to their site distribution
Figure 3: distribution of thyroid cancer patients according to their gender

Figure 4: distribution of thyroid cancer patients according to the histological grade.

Figure 5: distribution of thyroid cancer patients according to their tumor stage
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science and all experiments were carried out in accordance with approved guidelines.

References


Prevention and First Aid of Mechanical Airway Obstruction among Children: Supportive Stratiges for Mothers

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Abstract

Children considered as one third of today’s population and the whole future for us. For raising society being healthy, it is vital of having children being healthy. Mechanical airway obstruction is a communal and fatal fortune in group of pediatric age. It needs prompt treatment and recognition for avoiding outcomes as fatal. Despite aspiration of foreign body (FBA) could be event as threatening of life, it can be still preventable. The mostly significant risk factor for FBA is caregivers knowledge lacking. A pre/post quasi design of experimental was implemented in the outgoing study. Three hundred mothers as purposive were included in the current study. The study aim was to assess the supportive strategies influence for mothers in respect to prevention and first aid of mechanical airway obstruction among children. The work was performed in the outpatient pediatric department of El Sayed Galal University hospital. Tool I: data sheet as socio-demographic for children and their mothers, Tool II and Tool III: sheet of questionnaire in respect to knowledge of mothers besides reported practices of mothers. Results showed that the mean age of the studied mothers was (34.13±8.97), and more than three quarters of them (%76.7) were didn’t receive previous training program. Differences of significant were detected between post and pre supportive strategy in knowledge of mothers and reporting practices in prevention and first aid of choking that wasn’t affected by their socio-demographic characteristics (P value >0.05).

Keywords: first aid, mechanical airway obstruction, children, mothers, Supportive Strategy.

Introduction

“The children of today are the adults of tomorrow. Every child has right to grow up in a healthy environment. They deserve to inherit a safer, fairer and healthier world. There is no task more important than safeguarding their environment” 18. Choking is the introduction of a foreign object into a child’s airway that come to be blocked and minimizes or obstructs completely the flow of air to lungs. Obstructions mostly are clean through child coughing but in certain circumstances airflow is blocked completely, it might be no sounds for alerting others and a person might die in few minutes. Choking episode signs might including the person can’t speak (which ordinarily can), they are not able breathe, sounds wheezy breathing, silent attempts at coughing, decreasing consciousness levels, silent or quiet cough, cyanosis 10,4. Mechanical airway obstruction is a communal and fatal fortune in group of pediatric age. It needs prompt treatment and recognition for avoiding outcomes as fatal. Foreign body (FB) aspiration/inhalation is still death cause, usually in early childhood ⁴. Choking is respiration interruption via internal airway obstruction, typically via small toys or food in children being young. Such inhibits O₂ from passing to brain and lungs that causes damage to brain or death within 4 minutes. Children below 3 years age are particularly choking-vulnerable due to small airways. Parents must remember maintaining balance of constant between child overprotecting and freedom giving to him in his learning process environment hazards 12,8. Despite aspiration of foreign body (FBA) could be event as threatening of life, it can be still preventable. It might minimize via risk factors identifying and applying strategies for managing accordingly the factors of risk. The mostly significant risk factor for FBA is caregivers knowledge lacking. Strategies for choking prevention include education the prevention and the first aid for choking. It is clear that
nurse being pediatric has significant function in respect to education which delivered to parents for reduction and prevention chocking among children

**Study aim**

The study aims are:

- To identify the mothers’ knowledge level and reported practices regarding prevention and first aid of mechanical airway obstruction among children.

- To assess the supportive strategies influence for mothers in respect to first aid and mechanical airway obstruction prevention among children.

**Design of research:**

Time series quasi research experimental design (pretest-posttest) was used for the study.

**Sampling and sampling size**

At the current work, 300 mothers as appropriate sample along their children of age below 5 years was enrolled to examine the supportive strategies influence for avoidance and first aid of mechanical airway obstruction among children were selected from the pediatric outpatient department of El Sayed Galal University hospital. Randomized sample was selected by using a SPSS, for sample size determining; analysis of power was performed utilizing 0.05 as significance level, 0.95 as the power and affect 0.25 sizes. The needed size of sample gotten was 300 mothers along children below 5 years old.

**Considerations of ethics**

For ethical considerations before collection of data, approval was gained from the research scientific hospitals board, outpatient departments head and nursing faculty and University of Helwan. Informed verbal consent was given by mother before study participation.

**Setting:**

This work was performed in the department of pediatric outpatient hospital of El Sayed Galal University.

**Instruments:**

Three information collection tools:

**Tool I:** Characteristics as Socio-demographic for mothers including; education level, age, state of working …etc. and. Socio-demographic characteristics of children include: gender, and age…etc.

**Tool II:** Mothers’ knowledge about chocking. It comprised 6 questions of 12 as total score for knowledge of mothers.

**Tool III:**

**Part I:** reported mothers practice in respect to prevention of chocking, that including measurers checklist utilized in chocking prevention, 18 items which mothers should know to prevent chocking,

**Part II:** Mothers’ reported practice regarding first aid for chocking child, adapted checklist include standardized steps for first aid used in reliving of chocking.

**Part III: Strategies as supportive for prevention of chocking**

This part included booklet guide for mothers to avoid chocking among children less than 5 years.

2.7 Study as pilot

pilot was performed on 10% of sample size for clarity ensuring, tools applicability, and sample estimation size and the needed time for collecting data. The pilot sample study was omitted from size of total sample.

2.8 Reliability and validity

Tools were given in to 5 expert’s panel in different fields of nursing to test validity content. Reliability test was performed utilizing test of Cronbach to be reliable accepted on (Cronbach alpha was 0.82).

**Technique**

This current work performed on 3 phases:

1- **Phase of pre-paratory:** A review of previous and current, international, and national literature being related was done in several problems aspects utilizing articles, books, magazines, and periodicals.

2- **Planning phase:** the supportive strategy was developed, after an extensive literature review
considering mothers and their levels of understanding. After that, the training was conducted.

3- Implementation phase:

Before collection of data and conducting the study, official consent was gained from the research scientific hospital board, the pediatric outpatient head of the department of El Sayed Galal University hospital.

- Informed oral approval by mothers was given before study participation.

- At the opening interview, the researcher introduced herself to initiate communication line explaining supportive strategies purpose and nature. The study was performed in the morning from 9 Am to 2Pm.

- Developing session of training according to obtaining information from initial evaluation the researchers developing training sessions for improving knowledge of mothers and practice regarding prevention and first aid of mechanical air way obstruction among children.

- Every mother along her child was individually interviewed following study method and purpose explaining. Each interview approximately took 30 to 40 minutes to finish filling in tool of study, based on mother’s response and understanding.

- Collected data were started at Oct., 2018 till end of Apr., 2019.

4- Evaluation phase:

In the out patients, mothers met with researchers for evaluating the test of strategy as post supportive

Results

Table (1): Socio-demographic studied mothers Characteristics and their children distribution % (n= 300)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Characteristics of Socio-demographic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Age of mother:</td>
<td></td>
</tr>
<tr>
<td>&lt; 25 years</td>
<td>35</td>
</tr>
<tr>
<td>25 ≤ 30 years</td>
<td>105</td>
</tr>
<tr>
<td>30 ≤ 35 years</td>
<td>98</td>
</tr>
<tr>
<td>35 ≤ 40 years</td>
<td>47</td>
</tr>
<tr>
<td>≤ 40 years</td>
<td>15</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>34.13± 8.97</td>
</tr>
<tr>
<td>Mother’s Level of education:</td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>29</td>
</tr>
<tr>
<td>Read &amp; write</td>
<td>38</td>
</tr>
<tr>
<td>Preparatory education</td>
<td>43</td>
</tr>
<tr>
<td>Secondary education</td>
<td>82</td>
</tr>
<tr>
<td>High education</td>
<td>108</td>
</tr>
<tr>
<td>Child’s age (years)</td>
<td></td>
</tr>
<tr>
<td>≤ 2 years</td>
<td>209</td>
</tr>
<tr>
<td>3 ≤ 5 years</td>
<td>91</td>
</tr>
<tr>
<td>Mean age= 2.92 ± 1.15</td>
<td></td>
</tr>
</tbody>
</table>
Child’s gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>197</td>
<td>65.67</td>
</tr>
<tr>
<td>Female</td>
<td>103</td>
<td>34.33</td>
</tr>
</tbody>
</table>

Birth order of child

<table>
<thead>
<tr>
<th>Order</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>98</td>
<td>32.67</td>
</tr>
<tr>
<td>Two</td>
<td>142</td>
<td>47.33</td>
</tr>
<tr>
<td>Three</td>
<td>39</td>
<td>13</td>
</tr>
<tr>
<td>Four and more</td>
<td>21</td>
<td>7</td>
</tr>
</tbody>
</table>

Previous first aid for choking training

<table>
<thead>
<tr>
<th>Previous</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>70</td>
<td>23.3</td>
</tr>
<tr>
<td>No</td>
<td>230</td>
<td>76.7</td>
</tr>
</tbody>
</table>

History of child choking at home

<table>
<thead>
<tr>
<th>History</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>196</td>
<td>65.3</td>
</tr>
<tr>
<td>No</td>
<td>104</td>
<td>34.7</td>
</tr>
</tbody>
</table>

Table (1) illustrated that, more than one third (35%) of the studied mothers were aged 25 < 30 years old while only (5%) aged ≤ 40 years, with the mean age = 34.13 ± 8.97, according to education level, extra than one third (36%) of them were of high level of education. nearly three quarters were working, while more than three quarters of them (76.7) were didn’t receive previous training program related to prevention and first aid of mechanical airway obstruction among children, and about two thirds of them (%65.67, %65.3) were male and had history of child choking at home respectively. Additional than $\frac{2}{3}$ of enrolled children (%69.67) were less than three years of age where mean age = 2.92 ± 1.19.

Figure 1: Knowledge source of the studied mothers regarding first aid and prevention of mechanical airway obstruction among children, before and after supportive strategy (n= 300).

Figure (1) represented over half (53.33) of mothers studied their source of knowledge regarding prevention and first aid of mechanical airway obstruction among children from relatives, neighbors, and friends while only (14.67) from health care workers (doctors and nurses).
Figure (2) revealed that nearly hundred percent (90.3%) of the studied mothers had unsatisfactory knowledge score regarding prevention and first aid of mechanical airway obstruction while only (2.0%) of them had good knowledge before supportive strategy compared to more than half (55.7) after supportive strategy. It was found that there was highly differences of significant between mothers’ total mean knowledge score before and after supportive strategy at p. value < 0.001**

Figure (3) revealed more than 3 quarters (75.67%) of the mothers studied had unsatisfactory score of practices regarding prevention and first aid of mechanical airway obstruction while only (3.67%) of them had good practices before supportive strategy compared to nearly half had fair and good practices (45.67 and 47.33) respectively after supportive strategy. It was found that there was highly significant of differences between mothers’ total mean practices score before and after supportive strategy at P. Value < 0.001
Discussion

Currently, safety of child stays significant parent’s anxiety. Exposed children to risk of injury as they raise, children have a very limited capability to react rapidly and correctly in situation of emergency, that elevate chocking and death risk.

Results exposed that studied mothers mean age was 34.13± 8.97 years old. According to education level, extra than one third of them were had high level of education. Nearly three quarters were working, while more than three quarters of them were didn’t receive previous training program related to prevention and first aid of mechanical airway obstruction among children.

These findings go in the same line with Nour et al., (2017) who assess attitude, knowledge, and mother’s practices towards accidents of home among children at KSA reported which about 35.16% of the studied mothers had age ranged from 20 – 30 years. While dis agree with More than half of them were not working (58.59%), had University education (57.81%) and having more than 2 children aged 2 – 6 years (54.69%) and one fourth had previous training on first aid but dis agree related working as More than half of them were not working (58.59%).

The findings of the current study reinforced by El-Sabely et al (2014), who studied Education of mothers and their knowledge regarding prevention of home accident among pre-school children in rural areas in Governorate of Sharkia, revealed mothers age means of (34.1±9.6) years. In respect to level of education, 33.3% finished university level where more than 1/2 injured children (59.3%) were of 3years or lower, more than 1/2 of them (58.7%) were males. But dis agree related to status of occupation revealed that over 1/2 of mothers (58%) with no work.

The current results represented that over 1/2 half mothers their source of knowledge from relatives, neighbors, and friends while only (%14.67) from health care workers(doctors and nurses). This result supported by 13 who revealed that, Nearly two third of participants depend on social media as a source of knowledge about home accidents followed by doctor/nurse and the least sources were books and newspapers (17.97%). As well as agree with child El-Sably et al (2014), who revealed that over 3/4 of them gained their data from relatives, and radio and TV, then “doctors and nurses” (15.3%) , “part of curriculum” approximately (14%) whereas source being lowest was from “books” (6.7%).

The current results illustrated there was difference of significant between mothers’ total mean knowledge and reported practice regarding prevention and first aid of mechanical airway obstruction among children, before and after supportive strategy. Such results are in accordance with the proposed hypothesis and reinforced with Alazab,(2012) and Eldosoky,(2012) who concluded that in the pretest, mothers of deficit knowledge.

The current study illustrated that nearly hundred percent of the studied mothers had unsatisfactory knowledge score regarding prevention and first aid of mechanical airway obstruction while only (2.0%) of them had good knowledge before supportive strategy compered to more than half after supportive strategy. it was found that there was highly differences of significant between mothers’ total mean knowledge score before and after supportive strategy at p. value<0.001. This goes in the same line with Karyś , et al., (2016) who investigated knowledge of mothers of first aid administration to pre-schoolers in incidents of choking, in Poland revealed that 58% evaluated their knowledge being poor while 18% were disable to self-evaluate their skills as they were not sure that their knowledge was accurate.

This result supported by 18 who assessed the structured teaching programme effectiveness on mothers knowledge in respect to accidents prevention among pre-schoolers in designated Anganwadi , revealed that pre-test majority of mothers 75 (75%) had knowledge as average, 14 (14%) had knowledge as good and 11 (11%) had knowledge as poor, whereas in post-test indicated a marked increase in knowledge level, that all the mothers 100(100%) had good knowledge regarding prevention of accidents among preschooler’s. This findings are comparable with the study findings of 9 post-test mean knowledge score (24.14±2.01) was upper than the pre-test mean knowledge score (10.33±2.06) (11). Another study by 17 conducted in mothers (155) in Brazil where results showed a significant surge in knowledge regarding accidents prevention in children in all questions self-applied (p<0.05).
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Nursing and all experiments were carried out in accordance with approved guidelines.

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CT Scan Findings of COVID-19 in Patients with Fatty Liver

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Abstract

Purpose: COVID 19) is a respiratory disease caused by a member of the coronaviruses family, named (SARS-CoV-2), discovered in December 2019 in China. This study aims to assess the association of the simple fatty liver disease with the severity of the chest computed tomography (CT) scan findings in COVID 19.

Materials and Methods: It was a cross-sectional study that enrolled 322 patients with positive (RT-PCR) tests and chest CT scan findings. Patients with chronic lung and liver disease, alcoholics, and abnormal liver function tests excluded from the study. The liver CT scan density assessed, accordingly the patients divided into fatty-liver and non-fatty liver groups. The severity of lung CT changes compared between them, using Chi-square test.

Conclusion: COVID patients with fatty liver have more progressive changes in the chest CT scan. They have similar lung lesion distribution to non- fatty liver patients. Crazy paving lesions, septal thickening, Lymphadenopathy and plural effusion in fatty liver patients occurs in the same frequency as non-fatty liver patients

Keywords: CT scan, COVID 19, Fatty Liver.

Introduction

(COVID 19) is a respiratory disease caused by a member of the coronaviruses family, named (SARS-CoV-2), discovered in December 2019 in China. (¹, ²) The majority of patients have cough, fever, myalgia, with or without dyspnea. Certain groups of patients developed severe illness. (³) COVID-19 diagnosis depends on the real-time polymerase chain reaction (RT-PCR) test. (⁴) Chest computed tomography (CT) is used for early detection, assess severity, and for follow-up of the patients. (⁴-⁸) Typical findings appear when lung tissue early reacts to the insult as focal peripheral rounded ground-glass opacities (GGO), which may become more extensive, confluent, and may evolve to dense consolidation . (⁹) Other less common CT scan findings include linear and curvilinear opacities, vascular enlargement, and bronchial dilation. Pulmonary nodules, lymph node enlargement, cavities, and pleural effusions are rare findings. (⁶,¹⁰)

Findings associated with high mortality include extensive lung involvement and the presence of consolidations. (¹¹-¹⁵) As published in recent studies, patients with cardiovascular disease, diabetes, chronic kidney disease, and older age are at a higher risk of infection and prone to a graver outcome once infected. (¹⁶-
Non-alcoholic fatty liver disease (NAFLD) is another comorbidity that affects the severity of COVID-19. It is a chronic disease characterized by the presence of hepatic steatosis (intrahepatic lipid of more than 5% of liver weight) without a history of alcohol intake, with or without abnormal liver tests. It is the liver manifestation of the metabolic syndrome, which is characterized by the presence of obesity, insulin resistance, hypertension, and hyperlipidemia. NAFLD has a doubtful association with infections; recent studies reported an association with community-acquired pneumonia and a higher risk of evolving to severe disease in COVID-19 patients.

The study aims to assess the association of the simple fatty liver disease with the severity of the chest CT scan findings in COVID-19.

Materials and Methods

1. Study population: It is a cross sectional study done for 322 patients with COVID 19 at Azadi Teaching Hospital/Kirkuk city/Iraq, attending the CT scan unit for evaluation of their chests from march 2019- June 2019.

Inclusion criteria: adult Patients with positive CT scan findings and nasopharyngeal RT-PCR tests were enrolling in the study.

Exclusion criteria: Include patients with a known history of heart failure, pre-existing lung or liver disease, alcoholic persons, those with positive serology for hepatitis virus, and abnormal liver function tests. We collected the clinical information from the documentation of the clinicians.

2. Imaging: CT examinations without intravenous contrast medium achieved by a multidetector-row scanner (Siemens Brilliance), the energy level of 200–250 mA, and 120 kV. The patients imaged in the supine position. The slice thickness was 5 mm, images taken from the upper neck to the level of the umbilicus. The CT scan examinations in both lung and mediastinal windows evaluated by two radiologists with at least five years’ experience in CT scan imaging. Any differences of opinion resolved with consent. The CT examinations were done at 3-10 days from the onset of the symptoms.

3. CT interpretation: Liver density assessed to confirm the diagnosis of hepatic steatosis as liver attenuation less than 40 HU. The CT COVID changes attenuation classified as pure GGO, predominantly GGO), or consolidation (Fig 1b), and pure consolidation attenuation, while the degree of lung involvement assessment done as the following: both lungs divided into six regions, three equal zones for each one: upper, middle, and lower zones. Each zone graded into less than 5% involvement, 5-25%, 25–50%, 50–75%, and > 75% lung involvement, then the average percentage is calculated for each patient.

4. Statistical analysis: Continuous variables expressed as mean (± SD) and categorical variables as frequency (percentage). The lung lesion densities and extension compared between two groups differentiated according to their liver involvement by the fatty change. A probability (p) value of less than 0.05 was considered statistically significant. Version 17, SPSS software was used for statistical analyses.

This study approved by the Faculty of Medicine’s Research Ethics Committee/ Kirkuk University - Iraq. The individual data not explored in the study.

The patients gave verbal informed consent to participate in this study.

Figure 1. a, b. A 57-year-old woman with COVID-19, in the fatty liver group. Axial CT image (a) of the upper abdomen in abdominal window, average liver density is 11.9 HU (b) chest CT scan in the lung window, shows predominant consolidation in the both lower lobes.
Findings

Three hundred twenty-two patients (160 males and 162 females) with laboratory diagnosed COVID-19 had positive CT scan findings. Their age range was 18-70 years, and the mean was 50.25 (±13.1), 66 (20.5%) of them had fatty liver and 256 (79.5%) had normal liver density. (Table 1)

Table 1: Demographic criteria of the study sample.

<table>
<thead>
<tr>
<th>Category</th>
<th>Fatty liver</th>
<th>Non fatty liver</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>66 (20.5%)</td>
<td>256 (79.5%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>50.92(±11.44)</td>
<td>50.08 (±13.49)</td>
<td>0.32 (t =0.46) 1</td>
</tr>
<tr>
<td>Weight</td>
<td>81.42 ± 14.3</td>
<td>76 ± 8.4</td>
<td>&lt;0.0001 (t = 3.97)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male N=160</td>
<td>33</td>
<td>127</td>
<td>0.46 2</td>
</tr>
<tr>
<td>Female N=162</td>
<td>33</td>
<td>129</td>
<td></td>
</tr>
</tbody>
</table>

1Using student t test, 2using chi-square test. The result is significant at p < 0.05. 3Number.

In the fatty liver group, the CT scan findings were pure GGO in 24 patients (36.36%), predominantly (more than 50%) GGO in 12 (18.18%), predominantly consolidation in 25 (37.87%), and Pure consolidation in 5 (7.57%). While in the non-fatty liver group, the findings were pure GGO in 75 patients (29.29%), predominantly GGO in 90 (35.15%), consolidation in 69 (26.95%), and Pure consolidation in 22 (8.59%). There is no statistical difference in pure consolidation and GGO between both groups in contrast to mixed density lesions which is predominantly GGO in the non-fatty liver group (P=0.003) and predominantly consolidation in the fatty liver group (P=0.04). (Table 2)

Table 2 relation between the lung lesions and liver density.

<table>
<thead>
<tr>
<th>Category</th>
<th>Pure GGO1</th>
<th>Predominantly GGO</th>
<th>Predominantly consolidation</th>
<th>Pure consolidation</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty-liver</td>
<td>24 (36%)</td>
<td>11 (16.66%)</td>
<td>26 (39.39%)</td>
<td>5 (7.5%)</td>
<td>66</td>
</tr>
<tr>
<td>Non fatty-liver</td>
<td>75 (30%)</td>
<td>90 (35%)</td>
<td>69 (26%)</td>
<td>22 (8%)</td>
<td>256</td>
</tr>
<tr>
<td>p-value</td>
<td>0.26</td>
<td>0.003</td>
<td>0.04</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

Using chi-square test. The result is significant at p < 0.05. Ground glass opacity.

Patients whose lungs involved by 5-25% were statistically more in the non-fatty liver group (P=0.00011), while more extensive involvement (25-50 %) was more in the fatty liver group (P=0.00001). While they had no difference in >5% and 50-75% lung involvement. (Table 3)
Table 3 relation between the lung lesions extension and the liver density.

<table>
<thead>
<tr>
<th>Category</th>
<th>Lungs percentage involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;5%</td>
</tr>
<tr>
<td>Fatty-liver</td>
<td>14 (21.21%)</td>
</tr>
<tr>
<td>Non fatty-liver</td>
<td>54 (21.09%)</td>
</tr>
<tr>
<td>Non fatty-liver</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Using chi-square test. The result is significant at p < 0.05.

In both fatty liver and non-fatty liver groups, the distribution of the lesion were mainly peripheral (63.63% and 56.25%), in lesser degree diffuse in (33.33%) and (35.15%) and to the least was central (3.03%) and (8.59%). The lesions in both groups were bilateral rather than unilateral (87.87% and 86.71%) versus (12.12%) and (13.28%). There was no significant difference in the lesion distribution in both groups. (Table 4)

Table 4 Distribution of lung lesions in the both groups.

<table>
<thead>
<tr>
<th>Category</th>
<th>Fatty-liver</th>
<th>Non fatty-liver</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral</td>
<td>42 (63.63%)</td>
<td>144 (56.25%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Central</td>
<td>2 (3.03%)</td>
<td>22 (8.59%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Diffuse</td>
<td>22 (33.33%)</td>
<td>90 (35.15%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Unilateral</td>
<td>8 (12.12)</td>
<td>34 (13.28%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Bilateral</td>
<td>58 (87.87%)</td>
<td>222 (86.71%)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Using chi-square test. The result is significant at p < 0.05.

Other CT scan findings in the fatty-liver group were as the following: Crazy paving 24 (36%), Septal thickening 5 (5.57%), Plural effusion 22 (33.33%) and Mediastinal lymphadenopathy 1 (1.51%). While in non-fatty liver were 100 (39%), 10 (3.9%), 86 (33.56), and 5 (1.95%) respectively. There was no significant difference in the incidence of these findings between the two groups.

There were no pericardial effusion, cavitation, neither nodule incidence in this study.

Discussion

In our study, 66 (20.5%) had fatty liver density, which approximates the global prevalence, which is about 25.24%. This group of people had a chance to develop more severe and extensive lung changes in COVID 19, as they had predominantly consolidative lesions, in contrast to the non-fatty-liver group who had GGO more. COVID 19 damaged the lungs of the Fatty liver group more extensively (25-50 %) in comparison to the non-fatty-liver group as their lungs involved more in (5-25%). It was consistent with the result of a study done in China by Kenneth I. Zheng et al who stated that (fatty liver patients that were obese had more severe COVID-19 disease) as the fatty-liver group’s weight was significantly more in our study. (fatty-liver disease patients had significantly more recurrent infections regardless of coexistent metabolic syndrome) a statement proved by William Nseir et al. The fatty
liver patients had a higher risk of progressive COVID 19, a conclusion made out by Julie Lucifora et al. (28) This relation is probably due to the association of fatty liver change with low vitamin D serum levels, which in turn increases the susceptibility to infection and autoimmunity. (29, 30) In addition to the deficient innate immunity to the coronavirus representing by high M2 blood macrophages, which suppress the response to the infections. In both groups, the distribution of the lung lesions was more frequently peripheral and bilateral, and to a lesser degree had a diffuse behavior, the least distribution pattern was unilateral and central lung involvement. The result was in line with much of the studies. (2, 6, 10,12)

In both groups, like other papers, other CT scan findings were seen almost in the same percentage. About one-third of the patient had crazy paving lesions and septal thickening. Lymphadenopathy and pleural effusion were relatively rare. (6, 10)

Limitation of the study: We did not consider the patients who may have received COVID 19 treatment, which might affect their CT scan findings.

**Conclusion**

COVID patients with fatty liver have more progressive changes in the chest CT scan.

The distribution of the lung lesions in both fatty and non-fatty liver are similar.

Crazy paving lesions, septal thickening, Lymphadenopathy and plural effusion in fatty liver patients occurs in the same frequency as non-fatty liver patients.

**Conflict of Interest:** None.

**Funding:** Self funded.

**References**


Impact of Work Environment upon Respiratory Tract Health Problems among Workers at Textile Industries in Baghdad City

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Abstract

Objective: To evaluate the effect of the work environment on the respiratory health problem of workers in the textile labs in Baghdad city.

Methodology: The sample consisted of 100 workers working in the wool and cotton fabrics in the General Company for Textile Industries in Baghdad for the period from 3 Jan of 2017 to 20 of May 2018. A randomized structured sample of (100) workers was selected using the probabilistic selection method. The questionnaire was built for the purpose of the study and the reliability of the questionnaire was verified through a pilot study and then it was presented to (10) experts to establish its credibility. (20) Items in the questionnaire divided into three sections, namely demographic information, symptoms and signs, and finally diseases that affect the respiratory system. The data were collected using the questionnaire method and analyzed the data by applying statistical descriptive analysis, which includes frequency, the mean and the computational mean, and the deductive statistical analysis, which included the Kay square test.

Results: Results of the study show that the results of the study indicated that most of the workers suffer from frequent coughs due to allergies to cotton and wool (68%) with persistent respiratory infections. The study concluded that most of the workers suffer from persistent cough due to the sensitivity of the raw material that enters the textile industries namely cotton and wool. Recommendation: The study recommended that further studies be carried out with the possibility of carrying out an educational program for workers to reduce the occupational hazards of the respiratory system in textile factories.

Key words: Impact, Work environment; Respiratory tract Health Problems

Introduction

The occupational health initiatives were started in collaboration with WHO in 1950, first of them was the formulation of an official definition of “Occupational Health”. Accordingly Occupational Health was defined as “Promotion and maintenance of the highest degree of physical, mental and social well-being of workers in all occupations; the prevention among workers of departures from health caused by their working conditions; the protection of workers in their employment from risks resulting from factors adverse to health; the placing and maintenance of workers in an occupational environment adopted to his physiological and psychological capabilities and to summarize: the adaption of work to man and of each man to his job “. In simple words, it is concerned with health problems in relation to man & his general social, biological, chemical & physical environment[1]. The occupational risks are estimated to be the tenth leading cause of morbidity and mortality among the global risk factors. The cost of occupational disease burden is estimated to be 2-14 percent of the gross national product for various countries. The work-related risks were estimated to cause 7,75,000 deaths globally in 2000. Of this the leading causes were unintentional injuries (41 percent) followed by COPD (40 percent), and cancer of the trachea, bronchus or lung (13 percent) being the major contributors of deaths. The injuries causing 3,10, 000 deaths, the dust-related...
The number of deaths related to carcinogens is 2,43,000 and the work-related carcinogens account for 1,46,000 deaths. The textile industry is the second largest industry in the world after agriculture. In India, the textile industry significantly contributes to the foreign exchange earned by the country [2].

Respiratory diseases and illnesses, such as asthma, bronchitis, pneumonia, allergic rhinitis, and sinusitis, can greatly impair a child’s ability to function and are an important cause of missed school days and limitations of activities. Symptoms associated with both mild and severe manifestations of these respiratory conditions, such as cough, wheeze, congestion, chest pain, shortness of breath, respiratory distress, and death in the most extreme cases, are responsible for substantial morbidity and a large cost burden to families and society [3].

Methodology

A descriptive assessment design is carried throughout the study to assess the work environment among textile industry workers in Baghdad City from January 3rd 2017 to May 20th 2017. The permissions have been obtained from Baghdad (Textile Industry, Cotton and Woof Textile) to carry out the study. The study is conducted at two groups (General Company for Cotton Textile Industry and General Company for Woof Textile Industry) in Baghdad City. A non-probability (purposive) sample of 100 workers is selected from the general company for cotton textile industry and general company for woof textile industry in Baghdad City. A question has been constructed to achieve the aims of the study, which comprises three parts: the first part contains the demographic characteristics sheet which consists of 7 items and the second part contains 10 items and the third part contains 10 items. A pilot study is carried out from January 3rd 2017 to May 20th 2017 to determine the content validity and internal consistency split – half reliability of the study instrument. Reliability of the questionnaire is used to determine the accuracy of the questionnaire since the results showed very high level of the stability and internal consistency of the main study domains at the level of items of the questionnaire.

Spearman rank correlation coefficient

Content validity of the instrument is determined through panel of 9 experts. The experts are asked to review the instrument, and their responses indicate that all of the experts agree that 20 items of the instrument are clear and adequate for the measurement of the phenomenon underlying the study. Data collection through the use of the study instruments and the interview technique as means for data collection from February 4th 2017 to 20th March. Data are analyzed through the application of descriptive data analysis approach which includes frequency, percent, and total score and inferential analysis such as chi-square.

Results of the Study

With respect to subject of “Textile Type”, the studied sample is distributed similarly between Cotton and Wool sectors, and for each sector, they are accounted for 50(50%). Relative to subject of “Age Groups”, the studied sample is distributed similarly among the sex groups with no significant difference at P>0.05. On subject of “Educational level for fathers and mothers”, the results illustrated that most of the studied sample had good graduated levels, such that “Secondary, and Intermediate” degree, and they are accounted for 37(37%) and 24(24%) respectively, and statistically reported high significant differences at P<0.01. “Type of work”, the results that most of machine operators are the more half of the sample officers, and they are accounted for 56(56%), while most of workers are married they are accounted for 89(89%), and statistically reported high significant differences at P<0.01. Income, and socio-economic status illustrated that most of the studied sample had a moderate level, and accounted for 78(78%) with no significant difference at P>0.05 compared with different groups.
Table (1): Distributive of Sign and symptoms of the sample:

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>Yes</th>
<th>F</th>
<th>No</th>
<th>F</th>
<th>Mean</th>
<th>Asses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  I have difficulty breathing</td>
<td>53</td>
<td>53</td>
<td>47</td>
<td>47</td>
<td>0.80</td>
<td>N.S.</td>
</tr>
<tr>
<td>2  I have a feeling for cotton or wool</td>
<td>51</td>
<td>51</td>
<td>49</td>
<td>49</td>
<td>0.63</td>
<td>N.S.</td>
</tr>
<tr>
<td>3  I suffer from persistent cough</td>
<td>68</td>
<td>68</td>
<td>32</td>
<td>32</td>
<td>0.05</td>
<td>S</td>
</tr>
<tr>
<td>4  I suffer from the secretions of the respiratory system (ulcer)</td>
<td>56</td>
<td>56</td>
<td>44</td>
<td>44</td>
<td>0.02</td>
<td>S</td>
</tr>
<tr>
<td>5  sounding during breathing (dribbling)</td>
<td>33</td>
<td>33</td>
<td>67</td>
<td>67</td>
<td>0.81</td>
<td>N.S.</td>
</tr>
<tr>
<td>6  I have cases of discoloration in the lips</td>
<td>18</td>
<td>18</td>
<td>82</td>
<td>82</td>
<td>1.08</td>
<td>N.S.</td>
</tr>
<tr>
<td>7  Overwhelmed During Work</td>
<td>5</td>
<td>5</td>
<td>95</td>
<td>95</td>
<td>1.15</td>
<td>N.S.</td>
</tr>
<tr>
<td>8  Speed of breath</td>
<td>33</td>
<td>33</td>
<td>67</td>
<td>67</td>
<td>0.81</td>
<td>N.S.</td>
</tr>
<tr>
<td>9  The Deep Soul</td>
<td>2</td>
<td>2</td>
<td>98</td>
<td>98</td>
<td>1.08</td>
<td>N.S.</td>
</tr>
<tr>
<td>10 Change the sound</td>
<td>34</td>
<td>34</td>
<td>66</td>
<td>66</td>
<td>0.84</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

The results of this table shows all of items were non-significant except item (3,4) are significant.

Table (2): Distributive of Diseases of the sample:

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Yes</th>
<th>F</th>
<th>No</th>
<th>F</th>
<th>Mean</th>
<th>Asses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  I suffer from bronchial asthma</td>
<td>24</td>
<td>24</td>
<td>76</td>
<td>76</td>
<td>0.18</td>
<td>N.S.</td>
</tr>
<tr>
<td>2  bronchitis</td>
<td>88</td>
<td>88</td>
<td>12</td>
<td>12</td>
<td>0.23</td>
<td>H.S.</td>
</tr>
<tr>
<td>3  Bronchial fibroses</td>
<td>5</td>
<td>5</td>
<td>95</td>
<td>95</td>
<td>2.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>4  I suffered from pulmonary tuberculosis</td>
<td>8</td>
<td>8</td>
<td>92</td>
<td>92</td>
<td>2.02</td>
<td>N.S.</td>
</tr>
<tr>
<td>5  I suffer from sore throat</td>
<td>53</td>
<td>53</td>
<td>47</td>
<td>47</td>
<td>0.41</td>
<td>S</td>
</tr>
<tr>
<td>6  I suffer from pulmonary congestion</td>
<td>66</td>
<td>66</td>
<td>34</td>
<td>34</td>
<td>0.34</td>
<td>S</td>
</tr>
<tr>
<td>7  Continuous choking</td>
<td>5</td>
<td>5</td>
<td>95</td>
<td>95</td>
<td>2.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>8  Fibrosis of pulmonary vesicles</td>
<td>33</td>
<td>33</td>
<td>67</td>
<td>67</td>
<td>0.81</td>
<td>N.S.</td>
</tr>
<tr>
<td>9  I suffer from lung cancer</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>2.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>10 I suffer from bronchial cancer</td>
<td>1</td>
<td>1</td>
<td>99</td>
<td>99</td>
<td>2.09</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
The results of this table shows all of items were non-significant except item (5,6) are significant and highly significant item 2.

**Discussion of the Results**

Analysis of such characteristics reveals that the respects to subject of “Textile Type”, the studied sample are distributed similarly between Cotton, and Wool sectors, and for each sector are accounted 50(50%). Relative to subject of “Age Groups”, studied sample are Less than half the sample is slightly age-restricted between (41-45) years old, distributed similarly among the sex groups with no significant different at P>0.05. This is within the factory policy of making equality between men and women. On subject of “Educational level for workers”, results illustrated that most of studied sample had good graduated levels, such that “Secondary, and Intermediate” degree, and they are accounted 37(37%), and 24(24%) respectively, and statistically reported high significant differences at P<0.01. This category of workers who carry this certificate is the most class of workers working in this factory for low wages and carrying hard work. That most of machine operator the more half of sample officers, and they are accounted 56(56%), while most of workers are married they are accounted 89(89%), and statistically reported high significant differences at P<0.01.

Income, and socio-economic status illustrated that most of studied sample had a moderate level, and accounted 78(78%) with no significant different at P>0.05 compared with different groups. The study findings depict that most of these worker’s demographical characteristics, that the All pairwise correlations between education, income, and occupation were positive and were stronger for men than for women. The lowest correlation was between education and income, indicating that education is not a primary determinant of wage. Higher correlations were shown for education and occupation, suggesting that skills acquired during education may help determine occupation. Although correlations ranged from .23 to .67, their relatively low magnitude (highest adjusted R2 = 45%) indicates that the three dimensions are not redundant measures of SES.

2. **Discussion of the Subjects’ Sign and symptoms:**

This domain the all items were non – significant except I suffer from persistent cough (0.05) I suffer from secretion of respiratory system (ulcer) (0.02). One of the greatest risks to workers working in textile factories is respiratory infections and allergies in the airway and lung [5], Because it more exposure to dust and or other pollution in dust and or other pollution industries make if danger on the worker to developed respiratory health problem among worker.

3. **Discussion of the Subjects’ pulmonary diseases:**

Result of this table shows all items were non – significant except I suffer from sore throat (0.41) suffer from pulmonary Congestion (0.34) are significant and high significant item bronchitis (0.00). Because of the worker’s don’t wear the protective Cloth or PPE that protect them from the sore threat and pulmonary Congestion so they more exposure to it. The values, or for employees who show a decrease in pulmonary function on the after-exposure test. If the physician feels significant changes have occurred from year to year, or if the worker has complaints about breathing, six-month test will also be done. Employees who are below 60 percent of the predicted value on their breathing test will be sent to a physician for an evaluation. Employees will be furnished written information on the results of their examination.

4. **Discussion of the Relationship between Workers’ Knowledge and Their Demographic characteristics of the Study:**

Analysis out of this table indicated that significant relationship were reported between the subjects age and worker’s knowledge. Because of the more age in textile worker is (41-45) years and young additional too Long shift of work and direct exposure to cotton and wood this make these a going more have Vern ability to disease. Age differences account for between 0-5 percent to %2 of the overall explained Variation in the data when no other Variable are controlled, (the relation of age to workplace injuries). Table 4 shows that the significant relationship was reported out of these between the subjects gender and worker’s Know at textile work. This table focuses on productivity and earning for tow reason. Frist, a focus exclusively on labor force participation provide only a partial picture of women and men’s experience in the labor market. Second, despite significant progress in female labor force participation over past 25 years,
prevised and persistent gender differences remain in productivity and earning across different sectors and jobs. Men’s and women’s jobs differ greatly, whether across sector, industries, occupation, type of jobs. Or type of firms. Compared in other studies female have about 40 over world in 2007-2008. [6].

Non- significant relationship was reported out of this between the marital status and worker’s knowledge (table 4). Because of the socioeconomic state play important role in worker’s life. Spicily in young to make family or who or how have family and children to cover costs of education or other responsibilities. The significant relationship between educational level and worker’s knowledge. Because the women’s stay how educational level than men’s. The women’s more closed to machines of productive cotton and wood so the women have more Vern ability to disease. The significant relationship between type of work and worker’s knowledge. Because of worker’s have direct contact to machines so they have direct contact or exposure to wood and cotton dust and other factor, noise. more than those who work in office job mean not closed to machines. So the worker has high risk or Vern ability to disease dependent on type of work or type of machines. Also the duration of exposure to dust or there factor make worker’s develop health problem among respiratory system.

**Conclusion**

The study showed that the majority of workers who deal with cotton and wool suffer from persistent cough, and the study showed that the work environment has a direct impact on the respiratory system of workers especially that most suffer from bronchitis. The study found that older workers in the 41-45 age group are more likely to suffer from respiratory infections. Environmental factor of physical, biological, chemical, psychological, and social ones had critical impact upon the worker’s health status.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Effects of Addition of Melatonin and L-Arginine on Cooled Semen Parameter of Iraqi Local Breed Rams in Vitro


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Abstract

The current study was carried out on six Iraqi breed rams, aged between 2 to 4 years, during the period from Nov. 2018 to the Mar. 2019. Semen samples were collected from each ram with electro ejaculator at the morning, one ejaculate per week over a time of 16 weeks. Pooled semen for all rams were used to evaluate the characteristics of semen to avoid individual variation between the animals. The volume and the color of fresh semen was recorded directly, then semen was evaluated for mass and individual motility, live/dead percentage and sperm abnormalities. Semen samples were diluted 1:10 with a Tris-based extender, and divided into seven parts (each part 2ml), 0.1µmol, 1.0µmol & 3.0µmol concentration of melatonin were added to the T1, T2 and T3 respectively, L-arginine were added to the T4, T5 and T6 in 0.001µmol, 0.1µmol and 1.0µmol concentration respectively while T7 serve as a control without any addition. All treatments cooled at 4°C. Then semen parameters evaluated. It was concluded from this study that addition of 0.1 µmol melatonin significantly protected ram sperm cell from cold storage that induced negative effects on sperm function. However the addition of L-arginine have no beneficial effect on cooled ram semen.

Keywords: semen characteristics, melatonin, L-arginine, anti-oxidants materials.

Introduction

Artificial insemination (A.I) plays an important role in sheep industry. It participates in distribution of Superior genetic materials from a little numbers of rams to a large numbers of ewes (32). These distribution of genetic materials leads to improvement of pure bred ewes (5) via increase meat, milk and wool production (45). Mammalian and ram semen seems to be more susceptible and sensitive to oxidative stress resulting from reactive oxygen species (ROS) produced from metabolic activity of cellular components of semen during storage due to a high content of sperm cell membrane of unsaturated fatty acids phosphor lipid (4, 16, 20, 34). Although the oxidation is important for life, but it may cause a harmful effect due to the formation of free radicals that cause a damage to sperm cells (20). The oxidative stress may cause a reduction in reproductive performance of ram through its effect on characteristics of seminal fluid, due to production of ROS, which have a great role in lipid peroxidation in the sperm membranes with production of fatty acids peroxides that leads to a decrease in sperm motility and reduced their ability of fertilization (11). In order to reduce the effect of these free radicals or prevent their action, antioxidant either natural or synthetic were used (39, 44) Arginine is an amino acids of alkaline groups having a positive charge which includes; Lysine and Histadine, acts as antioxidant through its stimulation of Glutathione peroxidase which acts on Hydrogen peroxide (H2O2) and organic peroxide which is a site compounds resulted from metabolic activity of the body to water and oxygen ions. This peroxide when left to react with iron or copper ions it produced free radical of Hydroxyl that have a strong reaction (49). Arginine metabolism in the body resulted from the effect Arginase enzyme that leads to production of nitric oxide (No), ornithine and urea (47). It has been reported by many authors that treatment with arginine effect the male and female reproductive system.

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through the increase blood supply of genital organ and increase sexual desire (5, 31). Kaya et al. (25) observed that injection of arginine intramuscular increase sperm motility, sexual desire and accelerate ejaculation in the ram. The mechanism of arginine action act to increase sperm motility might be due to increase synthesis of ATP that supplied sperm with energy (33). Melatonin (N-acetyl-5-methoxytryptamine) or it may called dark hormone, secreted mainly from pineal gland, but it has been recognized to be synthesized in many other sites (36). It consists mainly from the amino acid tryptophane, and its receptors present in a numbers of tissues and organs of the body. It control or regulates daily and seasonal harmony of the biological activities in the body and increase sensitivity of leydig cells to interstitial-cell stimulating hormone (ICSH) (9). Also it controls many serious physiological events, including metabolism, sleeping, circadian rhythms and body temperature stability (12, 28, 43). Furthermore, it acts as anti-oxidant and anti-apoptotic substance (48) through its ability to remove different types of free radicals such as ROS, H₂O₂, OH⁻ and activate the production and catalyze of enzymatic antioxidant like; superoxide dismutase (SOD), Glutathione peroxidase (GOX) and Catalase (CAT) (27, 37, 39). Melatonin have the ability of enhancing body immunity (19). And has been used to boost the maturation of ova and to improve embryo development in many species, sheep, cattle and buffalo (2, 30, 46). Deng et al. (13) reported that treatment of Rams with melatonin increase the testosterone levels in the interstitial cells of the testes through increase Insulin like growth factor from sertoli cells which regulates synthesis and secretion of testosterone due to presence of melatonin receptors on sertoli cells membranes (15). It has been observed that addition of antioxidant such as melatonin or L-arginine protect sperm cells form harmful effect of ROS and improve sperm activity during its storage in unfrozen state (1, 6, 29). The objective of the Current study was to investigate the effect of addition melatonin and L-arginine Tris extender on cooled ram Semen in vitro.

Materials and Methods

The current study was carried out on six Iraqi local breed rams aged between 2-4 years, Presented in the farm of college of veterinary medicine, University of Fallujah Al-Anbar province during the period from November 2018 to the March 2019. The animals were fed alfalfa and hay the water was given in a free choice. All animals were healthy and treated for internal and external parasite and vaccinated with Co-Baghdad and Pox. Semen samples were collected from each ram with electro ejaculator (Electro jac5/a neogen company, U.S.A.) at the morning one ejaculate per week over a time of 16 weeks (sixteen samples program). Samples were taken and put it in a water bath at 38°C. Pooled semen for all rams were used to evaluate the characteristics of semen to avoid individual variation between the animals. 1ml of semen sample was taken to determine its parameters. Volume of the semen was directly measured by reading of graduated marks of collecting tubes. Color of semen has been visually evaluated according to Salisbury et al. (40). Mass motility has been done by putting a drop of fresh undiluted semen on a warm slide at 36°C and examined under light microscope supplied with heat stage at 100× magnification. Estimate the swirl grade according to Chenoveth et al. (10), the grades include Rapid swirl (very good), slower swirl (good), general oscillation (fair), sporadic oscillation (poor). One drop of fresh semen plus one drop of sodium citrate were taken to estimate the individual motility. Two smears of semen stained with eosin-nigrosin, were prepared (8) and used to determine the percent of dead/ alive and morphological abnormal spermatozoa (primary and secondary) according to Bielański et al. (7). Sperm concentration was calculated with hemocytometer chamber (40). Semen samples were diluted 1:10 with a Tris-based extender according to concentration of spermatozoa (Tris: 24.2gm, citric acid: 13.4gm, fructose: 10gm, glycerin: 64ml, egg yolk: 192ml, distal water up to one liter) described by Eidan et al. (17). Diluted semen was taken and divided into seven parts (each part 2ml), 0.1µmol, 1.0µmol & 3.0µmol concentration of melatonin were added to the T1, T2, T3 respectively, L-arginine were added to the T4, T5 and T6 in 0.001µmol, 0.1µmol and 1.0µmol concentration respectively while T7 serve as a control without any addition. All treatments cooled gradually via addition a piece of ice until it reaches 4°C within 2h. Semen parameters evaluated after dilution and cooling. Statistical analysis was applied using Tuckey’s - W procedure and chi-square test according to Steel and Torrie (42).

Resultand Discussion

The Characteristics of fresh semen of local Iraqi
rams in non-breeding season are shown in table:1. There were no statistical difference (P≤0.05) in semen parameters between rams in different ejaculates of the same ram. These results indicates that the semen is of good quality even when it collected in non-breed.

Table: 2 show semen parameters after diluted with Tris based extender with addition of melatonin and L-arginine in different concentration (0.1, 1.0, 3.0) μmol and (0.001, 0.1, 1.0) μmol respectively. There were a statistical differences (P≤0.05) in individual motility %, dead/ alive% and sperm abnormalities between 0.1μmol melatonin concentrations as compared with L-arginine or control treated semen. Similar observation has been reported by many investigators (6, 23, 26). The addition of antioxidant such as melatonin to ram semen (6) has been shown to protect sperm against harmful effects of reactive oxygen species (ROS) and improve sperm motility during sperm liquid storage or in unfrozen state.

Table: 3 showed the effect of addition of melatonin and L-arginine after cooling on the percentage of dead/ alive sperm, which showed a statistical difference (P≤0.05) between different treated semen. In the current study it observed that melatonin addition to the diluent in vitro enhanced the cooled preservation capacity of ram sperm in a dose of 0.1μmol and efficiently maintained sperm motility and other parameters over 48h of cooled storage. Melatonin is an indole derivative secreted rhythmically from pineal gland, plays an important role in the reproductive functions in mammals (37). Melatonin presents in seminal plasma, having multiple actions on different physiological process, as its metabolites are indirect anti-oxidants and powerful direct scavangers that protected sperm cells from free radicals raised by their metabolism (3, 24). Melatonin also modulating the glutathione activity to improve mitochondrial health state and functions (18). L-arginine is found to effect the reproductive process. O’Flaherty et al. (35) determined that L-arginine has a protective effect on spermatozoa against the sperm plasma membrane lipid peroxidation and enhances the cell metabolism. It has been reported that low concentration of L-arginine increase sperm motility, whereas high L-arginine concentration decreases sperm motility (21, 22). In addition, it has been found that nitric oxide synthesized from L-arginine might help or assist to induce acrosome reaction, sperm chemotaxis, and sperm egg interaction (22, 38). In current study the ineffectiveness of L-arginine on ram semen in vitro might be due to the high dose usage as explained by (21, 22, 29).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>After dilution</th>
<th>After cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Individual motility%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 0.1μmol</td>
<td>71.66 ± 2.80a</td>
<td>60.83 ± 3.51a</td>
</tr>
<tr>
<td>T2 1μmol</td>
<td>65.83 ± 2.47a</td>
<td>56.66 ± 2.78a</td>
</tr>
<tr>
<td>T3 3μmol</td>
<td>58.33 ± 2.54b</td>
<td>46.66 ± 2.10b</td>
</tr>
<tr>
<td>T4 0.001μmol</td>
<td>56.66 ± 1.92b</td>
<td>41.66 ± 1.66b</td>
</tr>
<tr>
<td>T5 0.1μmol</td>
<td>52.5 ± 1.55b</td>
<td>42.5 ± 1.11b</td>
</tr>
<tr>
<td>T6 1.0μmol</td>
<td>40 ± 0c</td>
<td>32.5 ± 1.11c</td>
</tr>
<tr>
<td>T7 Control</td>
<td>63.33 ± 3.04b</td>
<td>50 ± 2.23b</td>
</tr>
</tbody>
</table>

Values: Mean ± SE.
Different letter show statistical difference at (P<0.05).
Table: 3 show the percentage of dead sperm after dilution and addition of L-Arginine and Melatonin

<table>
<thead>
<tr>
<th>Treatments</th>
<th>After dilution</th>
<th>After cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>T1 0.1µmol</td>
<td>18.66 ± 0.91a</td>
<td>19.5 ± 0.92a</td>
</tr>
<tr>
<td>T2 1µmol</td>
<td>21.66 ± 1.22a</td>
<td>23.5 ± 0.92a</td>
</tr>
<tr>
<td>T3 3µmol</td>
<td>26 ± 0b</td>
<td>22.16 ± 2.16a</td>
</tr>
<tr>
<td>T4 0.001µmol</td>
<td>27 ± 0.44b</td>
<td>31 ± 0b</td>
</tr>
<tr>
<td>T5 0.1µmol</td>
<td>31.83 ± 0.9b</td>
<td>34.83 ± 0.74b</td>
</tr>
<tr>
<td>T6 1.0µmol</td>
<td>37.5 ± 2.72c</td>
<td>40.33 ± 3.33c</td>
</tr>
<tr>
<td>T7 Control</td>
<td>19 ± 0a</td>
<td>22 ± 0a</td>
</tr>
</tbody>
</table>

Values: Mean ± SE.
Different letter show statistical difference at (P≤0.05).

The results of table-4 showed that there was a statistical difference (P≤0.05) between different treatments and doses as compared with the control one. The lower percentage of sperm abnormalities were observed in melatonin treatment with different doses especially at 0.1Mmol. Similar observations have been made by (13, 14, 15). It has been reported that melatonin promotes development of haploid germ cells from early developing spermatogenic cells of sheep under in vitro environment (13). It has been found that melatonin acts as anti-oxidant through their ability to remove various types of free radicals such as ROS, H₂O₂, OH⁻ and superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) (37). The result of L-arginine treatments were disagreed with several authors (25, 33, 49). The ineffective results of L-arginine might be due the concentration applied or used that decrease sperm motility and increase abnormalities (21, 22).

**Conclusion**

It was concluded from this study that addition of 0.1µmol melatonin significantly protected ram sperm cell from cold storage that induced negative effects on sperm function. However the addition of L-arginine have no beneficial effect on ram semen.
Table: 4 show the percentage of abnormalities of cooled ram semen after dilution and addition of L-Arginine and Melatonin

<table>
<thead>
<tr>
<th>Treatments</th>
<th>After dilution</th>
<th>After cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Abnormality %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 0.1µmol</td>
<td>6.58 ± 0.42a</td>
<td>7.25 ± 0.25a</td>
</tr>
<tr>
<td>T2 1µmol</td>
<td>8.08 ± 0.42a</td>
<td>9.75 ± 0.25a</td>
</tr>
<tr>
<td>T3 3µmol</td>
<td>9.58 ± 0.42b</td>
<td>10.25 ± 0.25b</td>
</tr>
<tr>
<td>T4 0.001µmol</td>
<td>12.66 ± 0.67c</td>
<td>14 ± 1b</td>
</tr>
<tr>
<td>T5 0.1µmol</td>
<td>16 ± 0c</td>
<td>19 ± 0c</td>
</tr>
<tr>
<td>T6 1.0µmol</td>
<td>19.33 ± 0.33d</td>
<td>23.33 ± 0.33c</td>
</tr>
<tr>
<td>T7 Control</td>
<td>14.66 ± 0.33c</td>
<td>18.5 ± 0.5b</td>
</tr>
</tbody>
</table>

Values: Mean ± SE. Different letter show statistical difference at (P≤0.05).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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The Role of IL-25 and IL-35 in Amoebiasis

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Abstract

Background: Amoebiasis is a protozoon infection of the human intestine spread through the world, the most prevalent form of the disease is amebic dysentery which characterizes acute diarrhoea with observable blood and mucus in stools.

Aims: This article aimed to detects the role of IL-25 and IL-35 in the immune response against amebic dysentery.

Methods: This study was conducted in Thi-Qar province-Al-Nasiriyah city in Muhammad Al-Mousawi Hospital for Children, the study included collection of (60) blood samples from amoebiasis patients and (30) apparently healthy children at a period from September 2019 to March 2020 with the age less than one year to 15 years that divides to four age groups, the levels of IL-25 and IL-35 were determined by ELISA technique.

Results: The results indicate that the IL-25 and IL-35 concentrations in serum samples from amoebiasis patients were significantly higher when compared with that from healthy controls. The highest level of IL-25 was recorded in the third age group of patients with level 1677.2 ± 867.2ng /ml, compared with the high level in the third age group of control with level 450.40 ± 97.31ng /ml. Also, the findings indicates the highest level of IL-35 recorded in the second age group of patients with level 291.0 ± 62.3ng /ml, while the high level recorded in the first age group of control with level 8.246 ± 0.60 ng /ml.

Keywords: IL-25, IL-35, Amoebiasis, cytokine

Introduction

Amoebiasis is an infection of the human intestine by E. histolytica protzoen, also called amoebic dysentery is acute diarrhoea with observable blood and mucus in stools and the existence of haematophagous trophozoites in stools or tissues. Non-dysenteric amoebic colitis presents as recurring seizures of diarrhoea with or without mucus and no visible blood with the presence of E. histolytica cysts or non-haematophagoustrophozoites (1). E. histolytica stimulate both the innate and adaptive immunity such as the resistance of the mucosal barrier and lymphocytes of the class Cluster of differentiation 4 (CD4), Cluster of differentiation 8 (CD8) and the presence of amoeba antibodies such as immunoglobulin Class A secretory (SIgA) (2).

The cells in the intestine adhere to and distinguish the Gal/GalNAc lectin of parasites by (TLR)-2/4, as these cells work to send pro-inflammatory signals and leads to the formation of inflammatory cytokines, including IL-1β, IL-6, IL-8, IL-12, IFN-γ and TNF-α, these signals lead to attraction of the defending cells like: neutrophils and macrophages to the position of amoeba infection (3). IL-25 belongs to the IL-17 family; the name for the alternative is IL-17E. The protein contains 177 amino acids, which are secreted by many human cells, such as T cells, macrophages, masts, epithelium, dendritic and other (4). A study by (5) treating infected mice with intestinal IL-25 showed that it stimulates the immune response against Trichinella spiralis infection.

Also, observed that intestine IL-25 would be inhibited during CDI infection in humans and mice.
Therefore they concluded that an IL-25 induces eosinophil to defend against *Clostridium difficile* colitis CDI \(^{(6)}\). IL-35 was first known in 2007, has been added in the IL-12 family for similarity in the unique heterodimeric structure. It is mainly provided by CD4 Treg as well as by stimulated B cell and a smaller extent by motivated endothelial cells and monocyte cells \(^{(7)}\). Li and his colleagues enrolled in an interleukins study that IL-35 and IL-37 ratio are higher in inflammatory bowel disease (IBD) patients, while serum IL-35 and IL-37 level were suggestively low in the ulcerative colitis (UC) patients compared with healthy controls (HC) \(^{(8)}\).

**Material and Methods**

The study involved collection of 3 ml of blood for each samples from 60 children suffering from amebic dysenteriae and 30 samples from apparently healthy children as control with the age ranging from less than 1 year to 15 years during the period from September 2019 to march 2020 in Al-Musawe Hospital for children in Al-Nasiriyah city-Thi-Qar province-Iraq. Serum cytokine concentrations of IL-25 and IL-35 were determined in the serum samples by using enzyme linked immunosorbsent assay (ELISA) technique according to manufacturer’s instructions (BIO-TEC-China) using microplate spectrophotometric reader.

**Statistical Analysis**

All data of the present study were statistically analyzed by using Microsoft windows 10 Excel (version2010) and SPSS version 24 (ANOVA for Leas Significant Difference LSD and Independent T. test).

**Results**

The study showed higher level of IL-25 and IL-35 concentrations in patients who infected with amoebiasis in comparison to health control and recorded statistically significant difference in levels of IL-25 and IL-35 between all age groups of patients compared with corresponding age groups of health control at P. value < 0.05 (Tables 1 & 2)

### Table (1) Level of IL-25 for patient and control according to age groups

<table>
<thead>
<tr>
<th>Parameter Grups</th>
<th>No. of Cases</th>
<th>IL-25 M ± SD ng /ml</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>&lt; 1 year</td>
<td>18</td>
<td>1233.1 ± 405.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5</td>
<td>378.20 ± 48.97</td>
</tr>
<tr>
<td>Patient</td>
<td>1–5 years</td>
<td>47</td>
<td>1504.6 ± 643.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>14</td>
<td>444.91 ± 114.1</td>
</tr>
<tr>
<td>Patient</td>
<td>6–10 years</td>
<td>21</td>
<td>1677.2 ± 867.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>8</td>
<td>450.40 ± 97.31</td>
</tr>
<tr>
<td>Patient</td>
<td>11–15 years</td>
<td>11</td>
<td>1672.4 ± 700</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3</td>
<td>431.9 ± 157.1</td>
</tr>
</tbody>
</table>
Table (2) Level of IL-35 for patient and control according to age groups

<table>
<thead>
<tr>
<th>Parameter Groups</th>
<th>No. of Cases</th>
<th>IL-35 M±SD ng/ml</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>&lt; 1 year</td>
<td>18</td>
<td>240.6 ± 39.5</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5</td>
<td>8.246 ± 0.60</td>
</tr>
<tr>
<td>Patient</td>
<td>1–5 years</td>
<td>47</td>
<td>291.0 ± 62.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>14</td>
<td>6.746 ± 1.39</td>
</tr>
<tr>
<td>Patient</td>
<td>6–10 years</td>
<td>21</td>
<td>256.0 ± 72.8</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>8</td>
<td>7.170 ± 1.30</td>
</tr>
<tr>
<td>Patient</td>
<td>11–15 years</td>
<td>11</td>
<td>249.8 ± 52.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3</td>
<td>6.718 ± 1.59</td>
</tr>
</tbody>
</table>

Level of IL-25 and IL-35 According to Statues of Infection

According to status of infection the results of current study showed the high levels of IL-25 and IL-35 in triple infections, followed by double and single infections in comparison health control.

Table (3) Level of IL-25 and IL-35 according to statues of infections

<table>
<thead>
<tr>
<th>Parameters Infection Status</th>
<th>No. of Cases</th>
<th>IL-25 M±SD ng/ml</th>
<th>IL-35 M±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>1463.2 ± 645.4b</td>
<td>264.3 ± 62.4b</td>
<td>227.2 ± 28.7b</td>
</tr>
<tr>
<td>Double</td>
<td>1483.1 ± 605.7c</td>
<td>276.4 ± 60.7b</td>
<td>218.8 ± 26.6b</td>
</tr>
<tr>
<td>Triple</td>
<td>2631.0 ± 536.6d</td>
<td>305.1 ± 69.3c</td>
<td>263.0 ± 35.7c</td>
</tr>
<tr>
<td>Control</td>
<td>433.92 ± 104.0a</td>
<td>7.106 ± 1.34a</td>
<td>4.790 ± 0.7a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P. Value</th>
<th>LSD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.0001</td>
<td>172.86</td>
<td>30.16</td>
</tr>
</tbody>
</table>

Discussion

Level of IL-25 for Patient and Control According to Age Groups

The current study showed rising levels of IL-25 in all age groups and differences between them, significant differences were observed. These results indicate the impact of IL-25 with E. histolytica infection. In the present study, it is possible to find a role for IL-25 with amoebiasis by noting several things. High levels of IL-25 when we examined and compare patients of
amoebiasis with healthy control serum of all ages. This means that interleukins concentration does not affect with the patient’s age, but rather depends on increasing the number and concentration of parasites, also the immune status of patients, and the evidence is that the interleukin concentration increased in triple infections more than double and single infections.

This compatible with study by (9) pointed out that epithelial cells in the human intestine have the ability to produce IL-25, which has an important role in balancing defensive barriers in the intestine, as well as commensal bacteria in the intestine, which have the ability to stimulate intestinal epithelial cells to produce IL-25. The increase levels of IL-25 in this study may due to the ability of many types of cells to produce this cytokines such as T. cells, dendritic, macrophage, eosinophil and epithelial cells (4). IL-25 levels associated with increase the inflammation during intestinal infection because its ability to stimulate the production of some immunological mediators like: IL-8, chemotactic factors for neutrophil and induced inflammation also that IL-25 has been proved to enhance mucus production in intestinal (10), and production of Th2 cytokines such as: IL-4 which help in humeral defense mechanism against pathogens including parasite infection (11)(12).

So, IL-25 plays an important role in implicating immune response against amebiasis. As (13) mentioned that IL-25 provided protection from *E. histolytica* in an eosinophil-dependent, as demonstrated by abrogation of protection by depletion of eosinophils.

A role for eosinophil’s in amebiasis is show that decline eosinophil products (Charcot-Leiden crystals) exist along with trophozoites in the stool of patients with amebiasis (14). In addition (11) was also shown IFN-γ, IL-17(IL-25) contribute to vaccine-induced protection in murine studies, these findings suggest an important role for cell-mediated cytokine production in protection from amebiasis. Camelo and his colleagues found that IL-25-induced inflammation is typically characterized by elevated levels of type-2 cytokines which lead to pathological changes in the lungs and digestive tract, such as elevated serum IgE and IgG1, increased mucus secretion, and epithelial cell hyperplasia (15).

**Level of IL-35 for Patient and Control According to Age Groups and Statues of Infection**

The present study recorded the higher level of IL-35 in all age groups and statistically significant difference in levels of IL-35 between all age groups of patients compared with corresponding groups of health control. We showed distinctly the high level of IL-35 concentration with infection more than one parasite, where the highest infections were triple, double and single infections respectively. Also, the high level of IL-35 concentration does not depend on a specific age group, and we also recorded a rise in both sexes, as there are no statistical differences between them. This means that interleukins concentration does not affect by the patient’s age, but rather depends on the patients’ immune status.

The presence study agree with Cao and his colleagues in Chongqing, China, they found that IL-35 levels in serum samples from adult or child patients with sepsis was significantly higher compared with healthy controls and progressively increased according to sepsis severity (16). Also our study agree with study by Fonseca (17) in Mexico City recorded results suggest that down-regulation of inflammation in active Inflammatory Bowel Disease (IBD) patients might be based on the increased expression of IL-35 and IL-37. As in a study by (18) they found T cells that secrete IL-35 and have suppressive functions can be induced in the intestines of mice infected with the intestinal parasite Trichuris muris. Also agree with Choi found that IL-35 has an immunosuppressive effect on inflammation through induction of Treg cells and suppression of Th1 and Th17 (7). These results agree with (19) has been shown IL-35 in other chronic inflammatory diseases and parasitic/bacterial infections, where the inhibitory cytokines.

Depending on the results achieved in our study of patient’s children with amebiasis and comparing them with healthy children, and according to published research, we saw that the level of IL-35 rises in gastrointestinal infections, as it is considered an immune suppressant.

**Conclusion**

The cytokines under this study produces in high levels during the course of intestinal amoebic infections and they play important role in the development severity of the symptomatic form of amoebic dysentery.
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Appropriate Antibiotic Use for Community-Acquired Pneumonia in Inpatient Settings and Its Impact on 30-days Readmission and Mortality Rate

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Abstract

Background: Community-Acquired Pneumonia (CAP) is a lower respiratory tract infection with bacteria as the most frequent causative agent. Therapy for pneumonia includes appropriate antibiotic usage. Inappropriate antibiotic use supposedly increase 30-days readmission and mortality rate.

Objective: To evaluate the antibiotic use and the impact of appropriate antibiotic use on the 30-days readmission and mortality of CAP patients in inpatient non-ICU settings.

Method: A cross-sectional, analytic study was conducted. We collected data from Universitas Airlangga hospital’s medical record to obtain the details of antibiotic usage. Result were evaluated using the Gyssens algorithm. A chi-square test was used to identify the impact of appropriate antibiotic use on the 30-days readmission and mortality.

Result: A total of 90 patients with CAP fulfilled the inclusion criteria. One gram of ceftriaxone IV was the most prescribed antibiotic for therapy of CAP. The amount of appropriate antibiotic use is 85.6%. Five patients (5.6%) went through the 30-days readmission. There was no death reported. The statistical test between antibiotic use and 30-days readmission obtained \( p \) value=0.894 (\( p > 0.05 \)).

Conclusion: There was no significant impact of appropriate antibiotic use on the 30-days readmission rate and the mortality rate could not be assessed.

Keywords: 30-days readmission, antibiotic use, community-acquired pneumonia, Gyssens algorithm, mortality

Introduction

Antibiotic resistance can occur due to inappropriate antibiotic use. This unabating phenomenon increasingly becomes a problem in public health issues. Antibiotic resistance will rapidly increase along with inappropriate antibiotic use ¹⁻³. As methicillin was widely used in the 1960s, Staphylococcus aureus soon becomes resistant to the drug, treatment decision for MRSA infection can be challenging due to a change in its Penicillin Binding Protein (PBP) so that it is resistant to beta-lactam class antibiotics⁴⁻⁶. WHO stated that in 2013, at least 700.000 people died due to antimicrobial resistance to bacterial infection, malaria, HIV/AIDS, and tuberculosis. WHO also predicted that there will be approximately 10 million death due to antimicrobial resistance in 2050 ⁷. Appropriate antibiotic usage is substantial to the success of therapy. Inappropriate Empirical Therapy (IET) is said to be associated with a potentially worse outcome such as higher 30-day readmission rates and mortality rate⁸.

Lower respiratory tract infections, such as CAP, are the most common infection cause of death in the world and are the third cause of global death in 2008 ⁹. The mortality rate among CAP patients in non-ICU general
ward setting is reported to be around 2%. This rate increases in the ICU setting, >65 years, male, and patient with co-morbid diseases. This study was conducted to identify the appropriate antibiotic use in CAP patients and also to evaluate the impact of appropriate antibiotic use on the 30-days readmission and mortality of CAP patients.

Methods

This was a cross-sectional study that included all CAP patients (ICD code J18.9) admitted in inpatient non-ICU settings and were diagnosed with pneumonia in their first assessment examination. Patients were over 18 years old and hospitalized in the general ward of Universitas Airlangga hospital, Surabaya, East Java, from 1 January 2018 to 31 December 2018. We observed the antibiotic use in CAP patients through their medical records and collected data on patients’ characteristics, i.e., sex, age, admission date, and length of stay. We also collected data on patients’ antibiotic prescriptions, i.e., type of antibiotic, duration of therapy, dosage, route, timing, and interval time of prescription. Later we also observed the patients’ outcomes; 30-days readmission and mortality. The independent variable in this study was the antibiotic usage in CAP patients and the dependent variables will be 30-days readmission and mortality. We excluded those with incomplete medical records, patients with other infections, and patients who had been discharged against medical advice (DAMA). Total sampling was used in this study. Antibiotic usage was evaluated using the Gyssens algorithm and on the clinical practice guideline of the ATS/IDSA 2019 as well as the local guideline from Perhimpunan Dokter Paru Indonesia (PDPI) 2014. The Gyssens algorithm is a qualitative measurement to assess antibiotic usage and has been widely used in worldwide studies. The Gyssens algorithm will show the rationality of antibiotic usage based on grouping of 0—VI categories. A chi-square test was used to identify the impact of appropriate antibiotic use on the 30-days readmission and mortality.

Result

We collected 90 patients’ medical records who fulfil the inclusion and exclusion criteria of the study. Patients characteristic is shown in Table 1. The majority of the patient was female (57.8%), aged 45—64 years old (50%), and with the length of stay for 4 days (37.8%). Ceftriaxone (56.7%) was the most prescribed antibiotic for CAP, followed by levofloxacin (28.9%), ceftazidime (7.8%), meropenem (3.3%), cefixime (1.1%), cefotaxime (1.1%), and azithromycin (1.1%). Evaluation of antibiotic usage using the Gyssens algorithm will be expressed as ‘appropriate’ and ‘inappropriate’ and is shown in Table 2. Antibiotic use in 77 prescriptions were considered as appropriate (85.6%) while in 13 prescriptions were inappropriate (14.4%), according to Gyssens algorithm. The most common type of error was category IIIB (shortened duration) (11.1%). One prescription (1.1%) was classified as category IVA (alternating agent is more effective) and two prescriptions (2.2%) were classified as category VI (incomplete medical record), one of them showed no data of the interval time and the other contained no data regarding the timing of the prescription. It is reported that 5 of 90 patients (5.6%) underwent the 30-days readmission (Table 3), in which 3 of them were more than 65 years old and 4 of 5 them had co-morbid diseases (Table 4). All five patients who underwent the 30-days readmission had already been given the appropriate antibiotic prescriptions in their initial treatment (p=0.894) (Table 5). No mortality is reported in this study (Table 6).
Table 1. Characteristics of patient with CAP in 2018

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
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<td>18-24</td>
<td>2</td>
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<tr>
<td>25-44</td>
<td>7</td>
</tr>
<tr>
<td>45-64</td>
<td>45</td>
</tr>
<tr>
<td>&gt;65</td>
<td>36</td>
</tr>
<tr>
<td>Length of Stay (day)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
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<td>9</td>
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<td>Ceftriaxone</td>
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<td>Ceftazidime</td>
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<td>Meropenem</td>
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<td>Cefixime</td>
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<td>Cefotaxime</td>
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<td>Levofloxacin</td>
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<tr>
<td>Azithromycin</td>
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<tr>
<td>Duration of Therapy (day)</td>
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<td>2</td>
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<td>3</td>
<td>8</td>
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<td>30-Days Readmission</td>
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<td>85</td>
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<td>Mortality</td>
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<td>90</td>
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</table>
Table 2. Quality of antibiotic use expressed in Gyssens Category

<table>
<thead>
<tr>
<th>Gyssens Category</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>77</td>
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<tr>
<td>Appropriate</td>
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<td>II A</td>
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<tr>
<td>II B</td>
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<tr>
<td>II C</td>
<td>0</td>
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<td>III A</td>
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<td>IV C</td>
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<tr>
<td>IV D</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>2</td>
</tr>
<tr>
<td>Inappropriate</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

Note: 0: Appropriate; I: Inappropriate timing; IIA: Inappropriate dose; IIB: Inappropriate interval, IIC: Inappropriate route; IIIA: Prolonged duration; IIIB: Shortened duration; IVA: More effective alternating agent; IVB: Less toxic alternative agent; IVC: Less cost alternative agent; IVD: Narrower spectrum alternative agent; V: Inappropriate indication; VI: Incomplete medical record

Table 3. 30-days readmission in CAP patients

<table>
<thead>
<tr>
<th>30-days readmission</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>85</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 4. Risk Factors in Patients undergo 30-days readmission

<table>
<thead>
<tr>
<th>Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Co-morbid disease</td>
</tr>
<tr>
<td>Patient 1</td>
</tr>
<tr>
<td>Patient 2</td>
</tr>
<tr>
<td>Patient 3</td>
</tr>
<tr>
<td>Patient 4</td>
</tr>
<tr>
<td>Patient 5</td>
</tr>
</tbody>
</table>
Table 5. Crosstabulation between 30-days readmission and Gyssens category

<table>
<thead>
<tr>
<th>30-days readmission</th>
<th>Gyssens Category</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Gyssens 0)</td>
<td>(Gyssens I—VI)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>72</td>
<td>13</td>
<td>85</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>13</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 6. Mortality in CAP patients

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>90</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

Discussion

Types of Antibiotic

According to the 2019 ATS/IDSA Guideline for adults with CAP, two regimens are strongly recommended for CAP therapy in an inpatient setting; monotherapy of levofloxacin and combination therapy of beta-lactam and macrolide. Alternative for adults with both macrolide and fluoroquinolone contraindications is the combination therapy of beta-lactam and doxycycline.

Monotherapy of levofloxacin was prescribed in 26 patients (29.3%). A systemic review from 16 randomized control study reported that monotherapy of levofloxacin significantly caused less clinical failure, withdrawal, and diarrhea compared to the combination therapy of beta-lactam and macrolide.

Monotherapy of beta-lactam was prescribed in 63 patients (70%) and is the most prescribed antibiotic regimen for adults with CAP. Aside from combination therapy, monotherapy of beta-lactam can also be considered as an alternative for inpatients with non-severe CAP. A randomized controlled trial in 580 patients reported that monotherapy of beta-lactam failed to show noninferiority to combination therapy of beta-lactam and macrolide in moderately severe community-acquired pneumonia.

Another randomized controlled trial study also reported that there is no difference in mortality, complication, and length of stay between monotherapy and combination therapy in CAP patients.

One patient (1.1%) was prescribed with macrolide azithromycin as their empirical CAP therapy. Macrolide monotherapy is an antibiotic option for adults with CAP in outpatient settings, and is not recommended in inpatient settings. Macrolide works effective intracellularly and covers pathogens such as Gram-positive bacteria, Legionella, Mycoplasma pneumoniae, and Chlamydia pneumoniae. However, macrolide monotherapy should be given based on the resistance level and that is based on studies of macrolide failures in patients with macrolide-resistant S. pneumoniae. The rate of macrolide resistance among Streptococcus pneumoniae isolates in the United States is reported for >30%, a high level of resistance.

Duration of Therapy

It is reported that 79 out of 90 prescriptions (87.8%) were given for no less than 5 days, while 11 prescriptions (12.2%) were given for <5 days. According to the guideline by ATS/IDSA 2019, the duration of therapy should be guided by clinical stability (resolution of vital sign abnormalities), ability to eat, and normal mentation. Antibiotics should be given until clinical improvement
is achieved and is strongly recommended to be given for no less than a total of 5 days. Recent data supported that administering antibiotics for less than 5 days is barely sufficient. As most patients will achieve clinical stability in the first 48—72 hours of therapy, the total duration of therapy for 5 days will be sufficient for most patients and 7 days for CAP due to suspected or proven MRSA or *Pseudomonas aeruginosa* \(^{11,21}\).

**Appropriate Antibiotic Use**

There were 77 prescriptions (85.6%) classified as Gyssens 0, which means that the antibiotic use is appropriate. The result also shows that 10 prescriptions (11.1%) were classified as Gyssens IIIB, which means that the total duration of antibiotic therapy is too short (less than 5 days). Gyssens IIIB is the most common error found in this study. Another type of error is Gyssens IVA, which is found in 1 prescription (1.1%). Gyssens IVA implies that the antibiotic prescription is not appropriate because other antibiotics are more effective. One prescription in this category received azithromycin monotherapy. Azithromycin, which is a macrolide class of antibiotics, should be used together with beta-lactam antibiotics in empirical therapy for CAP in non-ICU inpatients \(^{11,12}\). The combination of beta-lactam and macrolide antibiotic therapy is said to provide better results than monotherapy which associated with a reduction in mortality and / or a shorter length of stay compared to the monotherapy with beta-lactam \(^{22}\).

The use of antibiotics as empirical therapy for CAP in this study can be concluded to have a rate of 85.6% of appropriate antibiotic use and 14.4% in other categories (Gyssens I-VI).

**30-days Readmission**

Five patients (5.6%) underwent readmission with the same diagnosis within 30 days after the patient was discharged. Early hospital readmission is a common and costly occurrence in elderly and high-risk patients. To improve the quality of care and to reduce unnecessary health expense, policymakers have made reducing 30-days readmission as a priority \(^{23}\). The incidence of 30-days readmission is often found in patients with CAP \(^{24}\). Zillberg *et al* stated that the 30-days readmission rate would increase if patients received inappropriate empirical therapy \(^{8}\). However, all five patients who underwent the 30-days readmission in this study had received appropriate therapy (Gyssens 0) on their initial CAP treatment. The statistical test between the 30-days readmission and the appropriate antibiotic use with chi-square test method obtained \(p\)-value = 0.894 (\(p > 0.05\)), suggesting that the incidence of 30-days readmission is not influenced by the appropriate antibiotic use and it can be concluded that the appropriate antibiotic use in this study has no significant impact on the 30-days readmission.

Undergoing the 30-days readmission while receiving appropriate therapy in the patients’ initial infection can occur due to some considerations. Factors such as age, immunodeficiency, malignancy, heart failure, and chronic conditions can increase the proportion of pneumonia cases that fail to have complete resolution. In other words, readmission for pneumonia may occur due to unavoidable causes. Patients with COPD, heart failure, diabetes, and malignancy will increase the proportion of readmission for pneumonia cases \(^{25}\).

In this study, 3 patients who underwent readmission were> 65 years old. Old age is related to a decrease in the general condition of the functional organ so individuals are more at risk of developing pneumonia \(^{26}\). Meanwhile, 4 patients had medical conditions other than CAP itself, such as malignancy, COPD, and heart failure. Those are known factors that cannot be avoided in readmission of CAP cases \(^{25}\).

**Mortality**

No mortality was found in this study. According to the guideline by PDPI in 2014, the mortality rate of CAP in inpatient settings is 5—20% and it will greatly increase in intensive care unit by more than 50%. However, it is also stated that the prognosis for CAP is generally good depending on several factors such as patient factors, causative bacteria, and the use of appropriate antibiotics \(^{12}\). We could not assess the impact of the appropriate use of antibiotics on mortality rate as there is zero mortality in both appropriate and inappropriate antibiotic use in this study.

**Conclusion**

Appropriate antibiotic use in adults with CAP in inpatient non-ICU settings of Universitas Airlangga
Hospital is 85.6%. The most common error in antibiotic usage is shortened duration (Gyssens IVA). The 30-days readmission rate is 5.6% and the mortality rate is zero. There was no significant impact of appropriate antibiotic use on the 30-days readmission and the impact of appropriate antibiotic use on mortality rate could not be assessed as there was no death in this study.

Ethical Clearance: This original research study had been approved by the Universitas Airlangga hospital Surabaya ethical committee in health research (3594/UN3.9.1/PPd/2019).

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Self funding.

References


Assessment of Knowledge and Perceived Practice on Using Eco-Friendly Toys among Mothers of Under Five Children

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Abstract

Introduction: Plastic is one of the most toxic substances in the world and there are several hazards of plastic usage. Plastic is a non-biodegradable product and do not decompose by biological actions of microbes. It takes about 1,000 years for plastic products to break down. They remain in the same state in the environment as we throw them whereas e-friendly are environmentally safe because the materials used to create the toys are natural, biodegradable and also recyclable.

Objectives of the Study: 1. To assess the level of knowledge on using eco-friendly toys among mothers of under five children. 2. To assess the perceived practice on using eco-friendly toys among mothers of under five children. 3. To determine the correlation between knowledge and perceived practice on using eco-friendly toys among mothers of under five children. 4. To find the association between socio-demographic variables and the level of knowledge and perceived practice on using eco-friendly toys among mothers of under five children.

Methodology: Survey study was conducted among 60 mothers with under five children at MK Pet, Tiruttani. Non probability convenience sampling technique was used to select the samples. Informed consent was obtained from all mothers, and made them be aware that information used only for research purpose. Structured interview schedule was conducted to elicit information from the participants.

Result: The inferences made are: Majority 40 (67%) had inadequate knowledge, 19 (31%) had moderate knowledge whereas least number 1 (2%) had adequate knowledge on using eco-friendly toys whereas 24 (40%) had fair perceived practice, 23 (38%) had poor perceived practice and only 13 (22%) had good perceived practice on using eco-friendly toys. There was a significant positive correlation between the mean score of knowledge 381 with S.D 6.35 and the mean score of perceived practice 724 with S.D 12. The calculated Karl Pearson’s coefficient of correlation r=0.07 was positively significant with low correlation at p < 0.05* level. there was significant association established with the type of family and mothers educational status at the level of P < 0.05* level. There was no statistically significant association found with the demographic variables of age of the mother, religion, education, number of children, occupation, monthly income with P < 0.05* level. The analysis revealed that there was significant association established with the religion and number of children variable at the level of P < 0.05* level. There was no statistically significant association found with the demographic variables of age, education, monthly income, type of family, number of children with P<0.05* level.

Key Words: Assessment, Knowledge, Perceived Practice, Eco-friendly toys, Mothers of Under Five Children

Introduction

Play is a child’s business and way of life. It is a legitimate right of childhood, representing a crucial aspect of children’s physical, intellectual and social development. All experts agree that children learn by playing and toys are the instruments that allow them to discover the world they live in. Ever since they are born,
toys motivate babies to use their feet and hands in order to discover forms, colours and sounds. Once babies know how to sit and crawl, toys incite them to be on the move. If their favourite toy is out of reach, the baby will use its muscles to try and grab it. Toys boost children’s creativity and they help them express their emotions (internal and external). Toys also motivate kids to take initiative, learn to negotiate and teaches them how to get better organized. Toys also promote children’s cognitive development by stimulating their concentration and memory skills (board games) and giving them the ability to solve problems creatively, which is key to their future autonomy (playing with building blocks). The Indian toy industry has shown tremendous growth and expansion potential in the domestic market that is estimated at about US $850 million and it generated 0.5 percent of the global market. Only 20% of the Indian market is served by Indian toy manufacturers while the rest is served by imported toys from different countries mainly from China and Italy.\(^\text{1,2,3}\)

We are living in a plastic planet. Plastics participate in human life from birth to death, and from waking to sleep, in objects of daily and specialised use alike. Plastic is a non-biodegradable product and do not decompose by biological actions of microbes. It takes about 1,000 years for plastic products to break down. They remain in the same state in the environment as we throw them. This, in turn, pollutes the land, sea and the atmosphere and one among them is plastic.

Here comes the question of safety. Toy safety is the practice of ensuring that toys, especially those made for children, are safe, usually through the application of set safety standards. In India also there is a move to make toys that meet global standards. But a large population lives in rural areas where toys which are sold are health hazard and injure the child in one way or the other. Choking is the number one reason for accidents, but chemicals such as lead can also cause developmental problems like behavioural disorders and sickness. Exposure to lead can affect almost every organ and system in the human body, especially the central nervous system. Lead is especially toxic to the brains of young children.\(^\text{2}\)

Indian markets are today flooded with Chinese toys which do not conform to any quality standards. Who and how should this be regulated? In many countries, commercial toys must be able to pass safety tests in order to be sold. It is time that we have a framework to protect the children and ensure that the toys available in the market are safe.

Eco-friendly products may seem to be more expensive, but long-term they are actually more cost efficient. Eco-friendly products tend to last much longer. These products are typically made from recycled materials and are sturdy, withstanding most drops, kicks, and dishwashers. Eco-friendly products may have a larger sticker price, but since they last longer, it is an investment that will pay off.

Plastics, for example, are known to have BPA, lead, and other harmful chemicals that can cause many different illnesses and diseases in both children and adults. For children, it can cause premature puberty, diabetes, stunted growth, and autoimmune disorders. Using eco-friendly products improves quality of life in terms of mortality, age, diseases, and illnesses. They ensure the safety of families and the planet.\(^\text{5}\)

Most of the children play with low-quality plastic toys which are highly dangerous. Plastic toys contain chemicals such as polyvinylchloride, phthalates, polystyrene, polyester and acrylic which are absorbed by the human body. They also alter hormone secretion. Hence, plastics are found to be highly carcinogenic and can be blamed for the growing incidence of childhood cancers as well as attention deficit hyperactivity disorder and learning disability. As health care providers, it is our prime responsibility to educate parents on the dangers associated with the use of plastic toys. So the researcher felt there is a need to conduct study on knowledge and perceived practice on using ecofriendly toys among mothers who is considered as primary care providers.\(^\text{7}\)

**Statement of the Problem**

“A descriptive study to assess the knowledge and perceived practice on using ecofriendly toys among mothers of under five children at MK Pet, Tiruttani”.

**Objectives of the Study**

- To assess the level of knowledge on using ecofriendly toys among mothers of under five children.
- To assess the perceived practice on using ecofriendly toys among mothers of under five children.
- To determine the correlation between knowledge and perceived practice on using ecofriendly toys among mothers of under five children.
To find the association between socio-demographic variables and the level of knowledge and perceived practice on using ecofriendly toys among mothers of under five children.

Null Hypotheses

- **NH₁**: There is no significant relationship between level of knowledge and perceived practice on using ecofriendly toys among mothers of under five children.

- **NH₂**: There is no significant association between socio-demographic variables and the levels of knowledge on using ecofriendly toys among mothers of under five children.

- **NH₃**: There is no significant association between socio-demographic variables and the levels of perceived practice on using ecofriendly toys among mothers of under five children.

Assumptions

- Mothers of under five children may have inadequate knowledge on importance of providing ecofriendly toys to their wards.
- Mothers of under five children may have poor perceived practice in buying ecofriendly toys in day to day life.
- Mothers of under five children may not have adequate understanding on impact of using plastics on the child as well as soil.
- Plastic toys impair child’s IQ level and it is a potent carcinogen.
- Indian parents and toy sellers have very less awareness about all types of toys and brands.
- It is difficult for the parents to differentiate among Indian and foreign toys for buyers.
- Money is not playing the major factor, choice of parent and child matters more. Many places, especially in rural areas, it is seriously difficult because they are not even aware of what is toxic and what is brand.
- Very less people were concerned about the toxics. Foreign Toy Industry is technology focused but toxics and attracts parents as well as children.

Delimitations

- The study was delimited to 1 weeks only.
- The study was delimited to under five mothers who were residing in MK Pet, Tiruttani.
- The study was delimited to under five mothers who are available at the time of data collection.
- The study focused only on perceived practice and the actual practice was not measured directly using observational check list in the mothers’ natural settings.

Projected Outcome

- This study will help the under five mothers to gain adequate knowledge and awareness on importance of ecofriendly toys.
- This study helps to alarm the mothers on ill-effects of buying plastic toys.
- This study help for the future reference for generating evidence based instructions on current scenario in using ecofriendly toys.
- This study may stimulate mothers converting from perceived practice into actual practice i.e., motivate them to go green (buying ecofriendly toys).
- Specific R&D for development of innovative and novelty toys and games will be set up to offer new green products as per fast changing needs/requirements of the domestic as well as export markets for toys.
- There will be a need to have a check over toxics available in toys.
- Advertisements will be telecasted to create awareness about toxics in toys and its ill effect.
- Strict laws will be formulated and executed if any toxic is found in toys.

Methodology

Research Approach

Quantitative research approach was adopted for this study to accomplish the objectives of the study.

Research Design

In this study, Non-experimental descriptive survey design was used.
Variables

Background Variables:
Mothers age in year, religion, type of family, monthly income in rupees, educational status of the mother, occupation of the mother, age of the child, number of child, number of plastic toys purchased in last one year, number of eco-friendly toys purchased in last one year, and how much spent for the toy.

Independent Variables:
The independent variable for the study was using eco-friendly toys

Dependent Variables:
The independent variables of the study were knowledge and perceived practice on eco-friendly toys.

SETTING OF THE STUDY
The investigator conducted the study in MK Pet urban area adopted by GRT College of Nursing, Tiruttani.

POPULATION OF THE STUDY

Target population
This is the population that the investigator had chosen to study and make generalization. The target population for the study was all the mothers with under five age children.

Accessible Population
The accessible population was all the mothers of under five age children who were living in in MK Pet, Tiruttani.

SAMPLE AND SAMPLE SIZE
In the present study, the mothers of under five aged who fulfilled the inclusive criteria were the samples.

SAMPLE SIZE
The sample size was 60.

CRITERIA FOR SAMPLE SELECTION

Inclusive criteria:
• The mothers who were willing to participate.
• The mothers who were residing in the selected urban area.

Exclusive criteria:
• The mothers who were not co-operative
• The mothers who were underwent educational programme on eco-friendly toys.

SAMPLING TECHNIQUES
Non probability convenience sampling technique was used to select the samples.

DEVELOPMENT AND DESCRIPTION OF THE TOOL
The instrument was developed and complied by the investigator with the guidance of experts and review of literature. The data collection questionnaire used in the present study had the following components:

Section A: Assessment of background variables
• Demographic variables of the mother: This included study participants age, religion, type of family, monthly income in rupees, educational status of the mother, and occupation of the mother.
• Demographic variable of the child: This included age of the child, and number of child
• Ecofriendly toys related factor variables: This included number of plastic toys purchased in last one year, number of eco-friendly toys purchased in last one year, and how much spent for the toy.

Section B: Structured Questions on Knowledge of Using Ecofriendly Toys

Section C: Structured Checklist on Perceived Practice of Using Ecofriendly Toys

CONTENT VALIDITY
Content validity of instrument was done by the panel of experts in the fields of Child Health Nursing, Nursing Research and Statistics. The experts’ suggestions were incorporated in designing the final tool for this study.

PILOT STUDY
Formal permission was obtained from the Head
of the Research Chairperson and Research Advisory Committee at GRT College of Nursing, Tiruttani. Individual permission was obtained from the mothers of under-five children. Pilot study was conducted in urban area at Tiruttani which was excluded from the main study. Brief introduction about the investigators and study was given. The confidentiality of the responses and the identity was assured. Pilot study was done on 6 samples. The pilot study aided the investigators to check the feasibility of conducting the main study in order to determine the statistical analysis and to assess the time required for data collection.

Reliability

Reliability of the tool was measured during pilot study using Karls Pearson coefficient of correlation’ test retest method for knowledge and inter rater method for perceived practice. The reliability r value were 0.78 for knowledge and 0.86 for practice. These values were very high thus making it a reliable tool for assessing the knowledge and perceived practice on ecofriendly toys among mothers of under five age children.

Data Collection Procedure:

A descriptive survey study was conducted among mothers of under-five children at MK Pet, Tiruttani after obtaining written permission from the head of the institution and Research Advisory Committee in GRT College of Nursing and Health Centre at MK Pet. The subjects were selected using convenience sampling technique. The data collection period was month. Data was collected from the mothers who were available in the time of study and accepted to participate in the study. Informed consent was obtained from all mothers, and made them be aware that information used only for research purpose. Structured interview schedule was conducted to elicit information from the participants. Explained the study participant’s rights to withdraw or withhold the information. Participants are provided with investigators contact information.

Data Analysis and Interpretation

Demographic Data

I. Frequency and percentage distribution of demographic of mothers of under five children

- With respect to the age of 27 (45%) were in the age group of 21-25 years, 26 (33%) were in the age group of 26-30 Years, 7 (12) were in the age group of 18-20 years and rest 6 (10%) were in the age group of 31 Years and above.

- In related to the religion dominantly 46 (77%) were Hindu, 10 (17%) were Muslim, 4 (6%) were Christians and none of them belonged to other community.

- In accordance with the educational status, secondary school education 20 (33%), primary education 17 (28%), graduate 14 (23%) and illiterate 9 (16%).

- In concern with occupational data most of them were home maker 40 (67%), 9 (15%) were private employee, 6 (10%) were daily wages and rest 5 (8%) were Government employee.

- Regarding the monthly income 22 (37%) were between 10001-15000, 6(27%) were getting below Rs.5000; 14 (23%) were Rs.50001-10,000; 8 (13%) were getting monthly income of above Rs.15000.

In related to type of family 35 (58%) were lives in nuclear family and rest 22 (37%) were belongs to joint family and only 3(5%) of the samples lives in an extended family.

In related to number of the children 27 (45%) were having one child, 24 (40) were having two children, 06 (10%) were having 3 children and 3 (5%) were having more than 4 children at home.

II. Frequency and percentage distribution of demographic of under five children

No=60

frequency percentage distribution of demographic and clinical variables of under five children. With regard to age of the child, majority of them 25(42%) belong to 1-2 years; 17(28%) are between 3-4 years; 12(20%) of them are above 5 years and 6(10%) are infants.

With regard to number of toys purchased in last one year, 21(34%) of the parents purchased only one toy of their wards; 19(32%) of them purchased 3 toys; 10(17%) of the parent purchased 4 toys and only 10(17%) of them purchased 5 and above toys for their children.

In concerned with the number of ecofriendly toys purchased in last one year, 25(42%) of them purchased 2 toys; 19(32%) were purchased 3 toys; 7 (11%) were purchased 4 toys; and only 9 (15%) were purchased 5 and above toys for their children.
In accordance with the amount spent on toys, majority of the parents 22(36%) were spend Rs.50-100; 14(23%) were spend between 10-50 rupees; 15(25%) were spend between 100-300 rupees and only 8(13%) spend Rs.300 and above.

III. Frequency and percentage distribution of Mothers of under five children based on their level of knowledge on using ecofriendly toys

The above figure 1 illustrates level of knowledge on using ecofriendly toys among mothers of under-five children.

The mothers of underfive children distributed as majority 40 (67%) had inadequate knowledge, 19 (31%) had moderate knowledge whereas least number 1 (2%) had adequate knowledge on using on ecofriendly toys.

IV. Frequency and percentage distribution of Mothers of under five children based on their level of perceived practice on using ecofriendly toys
The inferences made are, the mothers of under five children distributed as 24 (40%) had fair perceived practice, 23 (38%) had poor perceived practice and only 13 (22%) had good perceived practice on using ecofriendly toys.

V. Distribution of mothers based on their correlation between level of knowledge and perceived practice on using ecofriendly toys

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>r-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>381</td>
<td>6.35</td>
<td>r=0.07</td>
</tr>
<tr>
<td>Perceived Practice</td>
<td>724</td>
<td>12</td>
<td>p = 0.05</td>
</tr>
</tbody>
</table>

Significant at p<0.05*

The inferences made are

There was a significant positive correlation between the mean score of knowledge 381 with S.D 6.35 and the mean score of perceived practice 724 with S.D 12. The calculated Karl Pearson’s coefficient of correlation r=0.07 was positively significant with low correlation at p < 0.05* level.

VI. Distribution of mothers based on their association between socio-demographic variables and the level of knowledge on using ecofriendly toys

Association of level of knowledge regarding importance of play needs among parents of under five children with their selected demographic variables.

The analysis revealed that there was significant association established with the type of family and mothers educational status at the level of P < 0.05* level. There was no statistically significant association found with the demographic variables of age of the mother, religion, education, number of children, occupation, monthly income with P < 0.05 *level.
VII. Distribution of mothers based on their association between socio-demographic variables and the level of perceived practice on using eco-friendly toys

Depicts association between the levels of perceived practice regarding importance

Of using eco-friendly toys among mothers of under five children with their selected demographic variables.

The analysis revealed that there was significant association established with the religion and number of children variable at the level of P < 0.05* level. There was no statistically significant association found with the demographic variables of age, education, monthly income, type of family, number of children with P<0.05* level.

Conclusion

Play and childhood is inseparable. Play is any child’s business and it is their way of life which brings comprehensive development. Age-specific toys are used to comfort the child from the time of birth. Most of the children play with low-quality plastic toys which are highly dangerous. Young infants with too thin stage bite and suck on a lead-painted toy put them at risk of lead poisoning. Plastic toys contain chemicals such as polyvinylchloride, phthalates, polystyrene, polyester and acrylic are absorbed by human bodies and also alter hormones secretion. Hence, plastics are found to be highly carcinogen and it is evident that growing incidence of childhood cancers as well as attention deficit hyperactivity disorder and learning disability. As health care providers, it is our prime responsibility to educate parents on dangers associated with the usage of plastic toys. Eco friendly toys are environmentally safe because the materials used to create the toys are natural, biodegradable and also recyclable. We should create an awareness and make sure that every toy used by the children are eco-friendly, chemical free and safe.

Implications

The findings of the study has implications in different branches of nursing profession, i.e. nursing practice, nursing service, nursing education, nursing administration and nursing research.

Nursing practice

- The nurse role is to select the play materials according to age group of children with safety measures.
- The nurse practitioners can utilize ecofriendly play therapy to assist children in enhancing intellectual development and problem solving skills.
- The nurse can educate the children as well as their family regarding hazards of using plastic toys and its later effect on the child.

Nursing education

- Conference, workshops and seminars can be held for nurses to impart update their knowledge and positive practice towards using ecofriendly play articles.
- Nursing educator to update their knowledge and skills of providing ecofriendly play needs in various healthcare settings such as pediatric ward, ICDS and Centres should be given.

Nursing administration

- Nursing personal should be prepared to take a leadership role in educating parents regarding importance of play needs as well as ecofriendly toys. They should include their interest in educating parents during disseminate information about importance of play needs.
- The administrator can encourage the nurse for conducting research in various aspects regarding importance of ecofriendly toys
- The administrator can organize conference, workshop and seminar for nurses working in the pediatric ward
- The administrator should support the staff to conduct programmes on importance of ecofriendly toys.

Nursing research

- There should be more scope for research in this area to improve parents knowledge on importance of ecofriendly play needs. There is a need for extensive research regarding education techniques in order to improve the parents knowledge and in turn help bringing in positive and good practice regarding importance of using ecofriendly toys among mothers of under five children.
- The study will be useful for further reference.
- The results of the study encourage the parents to select suitable play materials according to age group of the children.
Encourage the nurses for conducting research in various aspects regarding importance of ecofriendly toys.

**Recommendations**

The study recommends the following

- A similar study may be conducted with large number of sample in different settings
- A comparative study can be conducted between rural and urban parents.
- A true experimental study with experimental and control group can be conducted.
- A similar study can be conducted through video teaching.
- A similar study can conducted to assess practice on important of ecofriendly toys in their daily life.

**Limitations**

The study has the following limitations

- Sample taken was only 60 mothers of under five children.
- Study was limited to assess the knowledge and perceived practice regarding importance of ecofriendly toys need among mothers of under five children.
- Duration of data collection was 1 week.
- The study assessed only parents of under five children.

**Conflict of Interest** – Nil

**Source of Funding** - Authors have no financial support to this project. The study was fully funded by authors.

**Ethical Clearance** – Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the participants. Informed the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential.

**References**

Canine-premolar Transposition, Family Pedigree and Related Dental Anomalies

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Abstract

Maxillary canine-premolar transposition is a rare and complicated dental anomaly that needs special consideration by the orthodontist. The aim of the present study was to investigate the clinical features of maxillary canine-premolar transposition and report the family pedigree to determine the mode of inheritance. A cross-sectional study was performed on 39 patients having maxillary canine-premolar transposition in which both clinical and radiographic examination were performed. Moreover, 112 family members were clinically examined looking for canine transposition. Variables such as location, sex and the presence of hypodontia and peg-shaped lateral incisors were investigated. The study found that unilateral transposition (89.7%) was more common than bilateral occurrence (10.2%) affecting the left side (64.1%) more than the right side (35.9%). Females were affected more than males (74.4% and 25.6% respectively). Congenitally missing teeth were reported in 12.8% and lower second premolar was the most commonly missing tooth. Peg-shaped maxillary lateral incisor was reported in 7.7% of the sample. Family pedigree confirmed the presence of a history of transposition or ectopically positioned canine in 15.3% of the sample suggesting an autosomal dominant inheritance of the trait. In conclusion this study suggested an association between genetic factors and maxillary canine-premolar transposition. Further studies are required using genetic testing to confirm the findings of the present study.

Keywords: Canine, Transposition, genetic, dental anomaly, peg-shaped, hypodontia

Introduction

Transposition of teeth is a rare condition which has a serious impact on the occlusion and is defined as a positional interchange between two adjacent teeth. Previously research provided important information on the prevalence of this anomaly which varies depending on the population featuring very low incidence (0.2-0.5%) 2-5.

Gender differences have been reported in different studies and are still unclear. Some research reported no gender difference 6, while others reported female predominance over males 4, though conversely some studies reported more affected males than females 7. Transposition is more frequently observed in the maxilla than the mandible 8, moreover canine-premolar transposition is the most frequent type 4, and unilateral presentation is more frequent than bilateral 9.

To date several theories have been proposed to be the causative factor of transposition such as trauma 10, early loss of teeth 11, positional interchange of the developing tooth bud 12, space deficiency 13 and genetic factors 3,4,10. Genetic aetiology has been confirmed by unilateral left-sided occurrence 1, hypodontia 7,11, retained deciduous teeth 11, peg-shaped lateral incisor 1. For these reasons genetic factors have been suggested to be the fundamental aetiology of dental transposition 1.
To our knowledge there have been few studies which reported various types of transposition and the associated dental anomalies beside reporting family pedigree looking for a family history of this dental anomaly. The aim of the present study was to investigate the clinical features of maxillary canine-premolar transposition and report the family pedigree to determine the mode of inheritance.

**Methods**

A total of 39 patients demonstrating maxillary canine and premolar transposition consisted of 10 males and 29 females (Table 1) with age range of 13-18 years at the time of diagnosis (mean age 13.7 years). No history of craniofacial anomalies or trauma was noted for all the participants. 112 family members were clinically examined looking for canine transposition and ectopic tooth position. The study population were collected from several private clinics in Baghdad city and the Orthodontic Department at the College of Dentistry/Baghdad University. The study was approved by a local committee in the Orthodontic Department at the College of Dentistry (Baghdad University).

The study focused on patients with maxillary canine-premolar transposition (unilateral or bilateral) who have not received any orthodontic treatment. The patients were clinically and radiographically examined using panoramic dental radiograph.

The following variables were reported:

1) The location of transposition, bilateral or unilateral.

2) Age at diagnosis.

3) The presence of missing teeth (hypodontia).

4) The presence of diminutive (peg-shaped) lateral incisor.

5) Family pedigree was recorded for each patients looking for a family history of transposition of teeth or ectopically erupted maxillary tooth.

Panoramic dental radiographs were available for all the patients at the time of diagnosis. They were used to confirm the presence of canine-premolar transposition, missing teeth excluding the third molar and peg-shaped lateral incisor. A lateral incisor was considered peg-shaped when the mesiodistal width at the cervical region was greater than the incisal edge. Descriptive statistics were used to define the characteristics of the study variables through the form of counts and percentages.

**Results**

A total of 39 patients with maxillary canine-premolar transposition (Figure 1) was examined both clinically and radiographically looking for the location and side of transposition and the presence of hypodontia or diminutive maxillary lateral incisor. The results revealed that unilateral transposition (n=35, 89.7%) was by far more common than bilateral transposition (n=4, 10.2%). In unilateral transposition left-sided occurrence (n=25, 64.1%) was more prevalent than right-sided (n=14, 35.9%). Unilateral occurrence was more common in females (n=25, 71.4%) than males (n=10, 28.6%). Similarly, left side occurrence was more common in females (n=17, 68%). Five of the 39 patients had congenitally missing teeth (12.8%) excluding the third molar. The lower second premolar was most frequently missing (three cases) followed by the maxillary lateral incisor. Peg-shaped maxillary lateral incisor was observed in three cases (7.7%), in some instances reduced sized lateral incisors were not recorded, since these small-sized teeth were not severe to fit the definition of peg-shaped teeth.

The family pedigree was also reported for other family members and first and second generation of the proband. The results revealed a family history of transposition or ectopically positioned maxillary canine was found in six families (15.3%). Two confirmed a similar condition in siblings while the other four mentioned an ectopically positioned tooth in a family member (Figure 2).
<table>
<thead>
<tr>
<th>Variable</th>
<th>(n, %)</th>
<th>(n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>35 (89.7%)</td>
<td>Male</td>
</tr>
<tr>
<td>Bilateral</td>
<td>4 (10.2%)</td>
<td>Female</td>
</tr>
<tr>
<td><strong>Side</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>25 (64.1%)</td>
<td>Male</td>
</tr>
<tr>
<td>Right</td>
<td>14 (35.9%)</td>
<td>Female</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (25.6%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>29 (74.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Congenitally missing tooth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>5 (12.8%)</td>
<td>Lower second premolar</td>
</tr>
<tr>
<td>Not present</td>
<td>34 (87.2%)</td>
<td>Maxillary lateral incisor</td>
</tr>
<tr>
<td><strong>Peg-shaped lateral incisor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>3 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>36 (92.3%)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1:** Intraoral photograph and panoramic radiograph showing transposition A) 12-year-old female with transposition in the maxillary left canine-premolar (encircled and arrowed). B) 16-year-old female with transposition in the maxillary left canine-premolar (encircled and arrowed).
Discussion

The present study focused on 39 patients with maxillary canine-premolar transposition who were clinically and radiographically examined. Previous studies reported that transposition most commonly occurred in the maxilla and canine-premolar teeth are most commonly involved [1,3,7,11]. Unilateral transposition was found to be more common than bilateral transposition which agree with other studies [1,3,8,11]. In bilateral cases, the same teeth were affected by the transposition which agrees with Al-Shawaf [8,15]. Left-sided occurrence was 64.1% which was comparable to what was reported by Peck et al [1].

Females were affected more by transposition when compared to males which was reported by most previous studies [1,8]. This phenomenon is not explained however, some reports of other anomalies such as cleft lip and palate found a left-side occurrence to be more common than right-side [16] and is dependent on the diagnostic procedure used. In this study we determined the prevalence of associated anomalies in patients with a cleft lip and/or palate, with a specific focus on cardiac anomalies. Materials and Methods: In this cross-sectional study, 526 patients with a cleft lip and/or palate admitted to the children’s referral hospital between 2006 and 2011 were evaluated. All associated anomalies were detected and recorded. Patient information collected included age, gender, type and side of cleft, craniofacial anomalies and presence of other anomalies, including cardiac anomalies. Data were analyzed using SPSS version 16. Results: Of the 526 patients enrolled in the study, 58% (305) Previous studies suggested the occurrence of maxillary canine-premolar transposition in girls more than boys [17]. Nevertheless, research has reported that most orthodontic practice patients are females [18]. For this reason, higher prevalence of females with transposition cannot be accounted for in comparison to males.

Figure 2: Family pedigree of a patients showing other affected family members. Empty circle=female, empty square=male, filled circle=affected female and arrow=proband.
The prevalence of hypodontia excluding the third molar is 12.8% and in the present study was twice as common as in the general population. Similarly, peg-shaped lateral prevalence was 7.7% twice as common as in patients with no teeth transposition. Most of previous studies reported higher prevalence of dental anomalies including hypodontia and peg-shaped maxillary lateral incisors when compared to the figures found in the present study which could be attributed to the sample size and ethnicity.

Interestingly, the present study reported the family pedigree for each patient, 15.3% confirmed the presence of ectopically positioned tooth or transposition. This finding could support the genetic aetiology of transposition together with the presence of dental anomalies. The pedigree suggested an autosomal dominant inheritance of the trait. Ely et al. reported that transposition is caused by both genetic and environmental factors based on the findings of their study. They supported their point by the fact that there was no previous study which reported a mutation in patients with transposition. Moreover, transposition may arise from developmental disturbances in the developing tooth dental follicle or genes that are involved in the development of dentition.

The limitations of the present study include no inferential statistics being performed due to the sample size. One of the strongest points in the present study is that the study focused on one type of transposition rather than different types of this anomaly which was seldom in previous studies.

Conclusions

The result of the present study suggested that genetics might play a role in the aetiology of maxillary canine-premolar transposition. This finding could be helpful to clinicians and can be supported by the utilization of recent technology including next-generation sequencing. Further large-scale studies are needed including larger sample and different types of transposition.

Source(s) of support: The study was self-funded.

Conflicting Interest: The authors declare no conflict of interest.

This study was approved by a local ethics committee in the Department of Orthodontics, College of Dentistry, University of Baghdad.

References


Potential of Chlorogenic Acid from Coffea canephora to Improving Innate Immunity System Components among BALB / c Mice

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Abstract

The aim of this study was to verify the phagocytosis activity which displayed in CD11b and B220 markers and also the markers of erythroid 2 nuclear factor related factor 2 (Nrf2) as a key regulatory transcription factor on various antioxidant gene expressions and Superoxide dismutase (SOD) as an antioxidant marker that related with protein. Nrf2 plays an important role to inhibit the ROS accumulation and eliminate free radicals. The active compound of chlorogenic acid in robusta coffee type (Coffea canephora) in Indonesian Coffee and Cocoa Research Center was used in three groups of mice in this study and it was gave different dose in each groups. The assessment samples were taken from the peritoneal fluid of mice than it was analyzed by using Flow Cytometry method to find phagocytosis function and antioxidant activity. The results of ANOVA statistical test was p <0.05 in all parameters, this mean that there was indicated that the active compound of chlorogenic acid in coffee was involved in natural immune system mechanism and it was seen in increasing of phagocytic activity and antioxidant levels.

Keywords: Coffea Canephora, chlorogenic acid, phagocytosis, antioxidant

Introduction

The natural immune system is a non-specific defence mechanism and quickly responds to antigen exposure in the body. The natural immune response is main weapon in host use to prevent or reduce pathogen replication in early stages of infection (1). The natural immune system as first body defence against infection (non-self) or tissue injury (damaged-self) with involves many cell and molecular components. Antibodies and complement was plays in Identification molecule solvent and cellular components plays in phagocytic cells (macrophages), antigen presenting cells (dendrite cells) and killer cells (NK cells). Another addition molecular is T and B lymphocyte cells who involve in natural immunity (2).

Phagocytosis process is one of an important aspect in natural immune response because it plays a role to inhibit an adaptive immune response (3), a highly-conserved mechanism occurs in first increasing before the development of other multicellular (4), the efficiency processes is eliminates pathogens attack and help in homeostasis repair process (5).

Macrophages and neutrophils are one of the key regulators in inflammatory process because it can produce reactive oxygen species (ROS) as a defence system to clean the microorganism components. ROS has two different ways. First, in high level as a as effector molecules against intracellular pathogens and second, in low level as a signalling messengers to inflammatory expression (6). The role of B lymphocytes as phagocytic cells is to produces ROS, and it happen

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because of the results of mitochondrial respiration due to high production of immunoglobulin or activation of NADPH oxidase complex (NOS)\(^7\).

The popularity of coffee drinks related with health effects and it was done in many studies on coffee and this provided some evidence such as coffee was reduced risk of cancer, improved neurological conditions, body metabolism and liver\(^8\). The phenolic compounds in coffee produced secondary metabolism which it have sentinel activity against free radicals and a role as antioxidants in body. The main phenolic compounds are phenolic acids, flavonoids and tannins. The phenolic acid family are include hydroxynamide and chlorogenic acids\(^9\). Based on this case, the researchers will assessed the phagocytosis activity by the description of CD11b and B220 also markers of nuclear factor erythroid 2 related factor 2 (Nrf2) as key transcription factor on various antioxidant gene expressions and Superoxide dismutase (SOD) as an antioxidant marker that was closely related with Nrf2 protein and it plays an important role to inhibit the accumulation of ROS and eliminating free radicals.

**Selection of coffee beans**

Four types of coffee beans were taken from several cities in East Java, Indonesia. Two types of arabica (Coffee arabica) and robusta (Coffee canephora) coffee beans were taken from one of the coffee bean suppliers on the Ijen Mount, and one of types of robusta coffee beans was taken from coffee plantations in Jember and one type of robusta coffee bean was from coffee planter in Sumber Asin Malang area.

**Measurement of chlorogenic acid levels**

The HPLC-LC-MS / MS test used to measured the levels of chlorogenic acid and it was applied on four different types of coffee beans, and the highest concentration of chlorogenic acid used in research process. Analyzes process used Thermo Surveyor HPLC LC-MS / MS system. A 2ul sample injected into LC at 16 °C, the column controlled at 30 °C, and the autosampler compartment was set in 16 °C. Columns used are Hypersil Gold specifications (50mm x 2.1mm x 1.9µm). UHPLC brand of ACCELLA type 1250 made by Thermo Scientific which consists of a vacuum degasser, quartener pump, thermostatic autosampler controlled by Personal computer through the x-calibur 2.1 program. Solvent A = 0.1% formic acid in Water and B = 0.1% formic acid in Acetonitrile. A mobile phase gradient with a speed of 300 µl / minute at a setting of 0.0-0.6.00 minutes 5% B, 0.6-3.0 minutes 75% B, 3.0-3.5 minutes 75% B, 4.0-5.5 minutes 5% B. The quantification compared with chlorogenic acid standard at 325nm of wavelength with ranged of 5-750 ng.

**Flow Cytometry analysis**

**Experimental animals**

The research subjects were male BALB / c mice aged 10-12 weeks with an average weight 20-25 grams, white skin color, active conditions and normal behavior, no visible anatomic. 24 mice were selected by using random method than they were divided into 4 groups and 6 mice in each group. Namely each group were: group 1 (healthy mice without coffee intervention), group 2 with coffee intervention per sonde of 0.5g / kgBW / day, group 3 with coffee intervention per sonde of 1.5g / kgBW / day and group 4 with coffee intervention per sonde of 2.5g / kgBW / day. The coffee intervention with different doses were carried out for 14 days, then the peritoneal fluid was taken in all mice and than it analyzed by using the Flow Cytometry method of phagocytosis function and antioxidant levels.

**Macrophages Isolation process**

The mice were dislocated on the neck, put on a sterile surgical board. The all part of mice body soaked with 70% EtOH and gave sterile injection of 10 ml PBS in an intraperitoneal. The ventral part (stomach) was tapped, then slowly taken peritoneal fluid by using a syringe and than the peritoneal fluid was put in a sterile 15ml propylene tube, than it centrifuged at 2500 rpm for 5 minutes at 10°C. The pellets were resuspended on 3 ml RPMI completed and homogenized, so in the final got macrophages.

**E. coli bacterial coloring and phagocytosis**

The 10\(^8\) cells / ml E. coli culture on sterile PZ media was transferred to a sterile 15ml propylene tube as much as 10ml than it was centrifugated 8000 rpm for 5 second at 4°C, than the supernatant was discarded, the pellets were resuspended with 0.5 ml sterile PBS and homogenized. The suspension heated on 80°C for 5 minutes. At normal temperature, the suspension add
5μl coloring solvent of carboxyfluorescein succinimydil ester (CFSE). Incubation process was did for 20 minutes at 4°C in the dark room. The stained bacterial suspension added to the macrophage cells that had been prepared before, than put in a 30mm petri dish, and cultured macrophage cells at 37°C CO₂ 5%.

**Flow Cytometry interpretation**

Macrophage cell culture carried out for 4 hours, than the suspension transferred to 15 ml propylene tube (avoided from direct exposure to light). After that, it was centrifuged in 2500 rpm for 5 second at 10°C. The supernatant removed, the pellets resuspended with 50μl working solution of specific antibodies (PE conjugated anti-CD11b, anti-B220, anti-superoxide dismustase (SOD) 1 antibody EP1727Y, and anti-Nrf2 antibody ab89443). Incubation process at 4°C for 20 minutes in dark room, then added 0.5 ml of PBS and homogenized. Then the suspension transferred to the FCM cuvette (polystyrene tube 12x75 mm) and read on a Flow Cytometry tool.

**Data Analysis**

Data analyzed by using SPSS with version 17 and the sample analyzed by using ANOVA and the significance data were continued test with Tukey’s test.

**Results and Discussion**

Chlorogenic acid HPLC analysis

**Table 1. The comparison of chlorogenic acid levels**

<table>
<thead>
<tr>
<th>Number</th>
<th>Kind of coffee beans</th>
<th>Sample</th>
<th>Weigh Spl (g)</th>
<th>Area</th>
<th>CONS. TKR (μg/ml)</th>
<th>F.P.</th>
<th>Weigh (μg/ml)</th>
<th>CONS. TKR (μg/ml)</th>
<th>Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Robusta of Jember</td>
<td>Spl_1_1</td>
<td>0.5109</td>
<td>21699</td>
<td>5.43</td>
<td>500</td>
<td>2,715.34</td>
<td>5,315.34</td>
<td>0.53</td>
</tr>
<tr>
<td>2.</td>
<td>Robusta of Sumber Asin</td>
<td>Spl_2_1</td>
<td>0.4244</td>
<td>26120</td>
<td>6.54</td>
<td>500</td>
<td>3,269.74</td>
<td>7,704.39</td>
<td>0.77</td>
</tr>
<tr>
<td>3.</td>
<td>Arabica BWI</td>
<td>Spl_3_1</td>
<td>0.3685</td>
<td>15087</td>
<td>3.77</td>
<td>500</td>
<td>1,886.92</td>
<td>5,120.54</td>
<td>0.51</td>
</tr>
<tr>
<td>4.</td>
<td>Robusta BWI</td>
<td>Spl_4_1</td>
<td>0.4059</td>
<td>10521</td>
<td>2.63</td>
<td>500</td>
<td>1,314.59</td>
<td>3,238.72</td>
<td>0.32</td>
</tr>
<tr>
<td>5.</td>
<td>Spray</td>
<td>Spl_5_1</td>
<td>0.4385</td>
<td>11062</td>
<td>2.76</td>
<td>500</td>
<td>1,382.50</td>
<td>3,152.79</td>
<td>0.32</td>
</tr>
</tbody>
</table>

The results of HPLC analysis showed that robusta coffee beans in area of Sumber Asin Malang was the highest levels of chlorogenic acid.
Figure 1. Graph of Chlorogenic Acid Fractionation

Results of Phagocytosis and Antioxidant Activities

Table 2. Results of the Quantification of Total (%) CD11b, B220, SOD, NRF2 by using ANOVA Test

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>N Mean ± SD</th>
<th>T1 Mean ± SD</th>
<th>T2 Mean ± SD</th>
<th>T3 Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b(%)</td>
<td>6</td>
<td>13.48±1.90  b</td>
<td>16.40±3.99  b</td>
<td>2.00±0.35   a</td>
<td>3.35±1.05   a</td>
<td>0.00</td>
</tr>
<tr>
<td>B220 (%)</td>
<td>6</td>
<td>14.38±1.91  a</td>
<td>23.65±4.28  b</td>
<td>16.41±2.64  a</td>
<td>34.22±6.64  b</td>
<td>0.00</td>
</tr>
<tr>
<td>SOD (%)</td>
<td>6</td>
<td>14.07±2.97  ab</td>
<td>20.28±1.75  c</td>
<td>10.61±2.96  a</td>
<td>17.53±3.9  bc</td>
<td>0.00</td>
</tr>
<tr>
<td>NRF2 (%)</td>
<td>6</td>
<td>12.91±2.59  a</td>
<td>12.71±2.67  ab</td>
<td>8.9±3.18    ab</td>
<td>14.63±2.44  b</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Note: * significant at α = 0.05, the ab superscript showed that there was no differences in each groups (ANOVA). n = number of samples, SOD = Superoxide dismutase, NRF2 = nuclear factor erythroid 2 – related factor 2, N = mice without treatment, T1 = mice with treatment 1 (0.5 mg coffee powder), T2 = mice with treatment 2 (1.5 mg coffee powder), T3 = mice with treatment 3 (2.5 mg of coffee powder).
The results of this study showed that there were significant differences in the amount (%) of CD11b, B220, SOD, NRF2 with ANOVA test. In T1 group (mice with treatment 1 (0.5 mg of coffee powder), the percentage of CD11b was higher than normal group. T2 and T3 groups were significantly different from the normal group. The highest percentage of B220 was in T3 group of mice with treatment 3 (2.5 mg of coffee powder). The highest percentage of SOD was in T1 group and the highest percentage of NRF2 was in T3 group and Tukey’s test results found that there were significant different of each groups with normal group.

Nuclear factor-Erythroid-related factor 2 (Nrf2) is a transcription of the basic leucine zipper redox-sensitive factor, namely pleiotropic proteins of regulate basal and induce basal expression of antioxidants and various other genes related to cell protection through binding of sequence enhancers (enhancers) and known as elements response of antioxidants. In normal condition, the Nrf2 level will be at low level but in stressful conditions such as oxidative stimuli, the Nrf2 will increases the transcription activation in targets that undergo protective conversion against various stress-inducing environments (10).

There was no differences of the Nrf2 level in each group, it mean that there was no environmental stress, but the active compound of Chlorogenic acid gave a different responses in each intervention group and described a response toward antioxidant function of Chlorogenic acid in coffee.

In this study, different doses were gave in each group of mice. There were differences of SOD enzyme in normal group and intervention group, there was higher antioxidant activity in low dose group compared with other groups. SOD as an endogenous antioxidant enzyme is a protein that acts as a first line of defense against ROS which clean superoxide radicals. The formation of ROS can find in a removing the potential of oxidants to become relatively stable compounds (11).

Figure 3. Phagocytosis by using Flow Cytometry method a). Untreated mice expressed that CD11b and B220 b molecules). Mice with treatment 1 (0.5 mg of coffee powder) express CD11b and B220 c molecules). Mice with treatment 2 (1.5 mg of coffee powder) expressed CD11b and B220 d molecules). Mice with treatment 2 (2.5 mg of coffee powder) expressed CD11b and B220 molecules.
CD11b is an integrin component that mediates monocytes, macrophages and granular cells to attach by using iC3b Opsonization which leads to phagocytosis, neutrophil aggregation and Chemotaxis. The expression of markers is related with T cell activation. Chlorogenic acid compounds as part of polyphenols which are present in large concentrations in coffee beans and it was proved in various clinical studies to reduce the risk of various diseases (12). In this study, the increasing of CD11b expressed that medium doses and high doses can be seen as a response to active compounds, especially Chlorogenic acid in coffee.

B220 is a CD45 isoform and it expressed in all mice B lymphocyte cells and as a subset of B lymphocyte cells in human, as a protein tyrosine kinase with various isoforms that regulate activation through a range of T and B lymphocyte cell surface receptors, as well as at cytokine receptors. CD45 known as B220, Ly-5 and T200, this mean that it is a member of the tyrosine phosphatase (PTP) protein family with a molecular weight of 220kDa. The PTP family has a function as a signaling molecule in regulatory process on cell differentiation, cell division and development. CD45R is the most common form of protein and it obtained by excess glycosylation when it expressed on B lymphocytes. NK cells (BioRad). In general, this study found that in intervention group (medium and high doses) were significantly different with normal group. Active compounds, especially Chlorogenic acid in coffee, are able to induce the activity of B lymphocytes as part of the natural immune system related with the ability of cells to internalize the phagocytosis process (13).

**Conclusion**

This study proved that the active compound of Chlorogenic Acid in Robusta coffee beans (Coffea canephora) which taken from Sumber Asin Malang area was the higher levels and it gave an effect in every component of natural immune system including phagocytosis mechanisms. The increasing levels of antioxidant was related to the neutralization mechanism of reactive oxygen species (ROS).

**Ethical Clearance:** Ethical Clearance of this study was obtained from the ethics commission of Medicine Faculty, Brawijaya University.

**Conflict of Interest:** There was no conflict of interests regarding the publication of this study

**Source of Funding:** The funding source of this study was supported by Directorate of Research and Community Service, Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education (Kemenristekdikti).

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